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Guarana (*Paullinia cupana*) ameliorates memory impairment and modulates acetylcholinesterase activity in Poloxamer 407 induced hyperlipidemia in rat brain

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Abstract

Hyperlipidemia is a risk factor for the development of cognitive dysfunction and atherosclerosis. Currently, the natural compounds have received special attention in relation to the treatment of diseases due to their low cost and wide margin of safety. Thus, the aim of this study was to determine the possible preventive effect of guarana powder (*Paullinia cupana*) on memory impairment and acetylcholinesterase (AChE) activity in brain structures of Poloxamer-407 induced hyperlipidemic rat. Adult male Wistar rats were pretreated with guarana 12.5, 25 and 50 mg/kg/day and caffeine (0.2 mg/kg/day) by gavage for a period of 30 days. Simvastatin (0.04 mg/kg) was administered as a comparative standard in this study. Hyperlipidemia was acutely induced with intraperitoneally injection of 500 mg/kg of Poloxamer-407. Memory test and evaluation of anxiety were performed. The cortex, cerebellum, hippocampus, hypothalamus and striatum were separated for the assessment of acetylcholinesterase activity. Our results revealed that guarana powder was able to reduce the levels of TC and LDL-C in a manner similar to simvastatin and partially reduced the liver damage caused by hyperlipidemia. Guarana was able to prevent changes in the activity of AChE and improve memory impairment caused by hyperlipidemia. Guarana powder may therefore be a source of promising phytochemicals that can be used as an adjuvant therapy in the management of hyperlipidemia and cognitive disorders.

Keywords: Caffeine, Hyperlipidemia, Memory, *Paullinia cupana*, Simvastatin

1. Introduction

Hyperlipidemia and hypercholesterolemia are major risk factors in the incidence and pathogenesis of some degenerative diseases including atherosclerosis, obesity cardiovascular disease and Alzheimer's disease (AD) [1 - 3]. Cholesterol plays crucial role in the development and normal functioning of the central nervous system (CNS). It maintains neuronal plasticity [4 - 6], transports synaptic vesicle along microtubules [7] and participates in neurotransmitter release [6]. The brain is rich in cholesterol and the major component of myelin; present in neuronal membranes, glial cells and localized in lipoprotein particles [8]. Peripheral and brain cholesterol pools are separated by the blood brain barrier (BBB) and are independently regulated [9]. Brain cells maintain cholesterol homeostasis by regulation of cholesterol synthesis and cholesterol uptake through apolipoprotein E (ApoE)-related receptors [9, 10].

The transport and redistribution of cholesterol in CNS cells are due to the lipoproteins presents in the cerebrospinal fluid [11]. The (ApoE), richly present in brain lipoproteins appears to be involved in the transfer of cerebral cholesterol, cholesterol rich regions to the regions with small quantities of cholesterol [12]. Cholesterol is synthesized *in situ* in the brain and a small quantity is present in the plasma. Cerebral renewal can occur by conversion of cholesterol to oxysterols; the compounds that can cross the BBB [12]. However, the poloxamer 407 (P407) is a ubiquitous manmade surfactant and non-ionic detergent, that across the BBB [13, 14]. It was observed that P407 induced the hyperlipidemia in experimental animals after parenteral administration [15]. Typically, cholesterol and triglycerides increase within 36 hours, of a single intraperitoneal injection of 500 mg/kg of P407. Poloxamer 407-induced hypertriglyceridemia and hypercholesterolemia are well-documented phenomena. Hypertriglyceridemia; characterized by elevated triglyceride levels are attributed to inhibition of lipoprotein lipase while hypercholesterolemia was linked to indirect stimulation of the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis, and decreased low density lipoprotein (LDL) receptor expression in all synthesizing cholesterol cells [15 - 17]. Cholesterol regulates biophysical membrane properties such as fluidity, permeability, and rigidity. The interactions between cholesterol and polar phospholipids can locally increase lipid order. This leads to the formation of dynamic membrane domains that contribute to the regulation of key cellular processes, such as receptor signaling, endocytosis and neurotransmission [18 - 19] , which can give rise to mood

and anxiety disorders [20]. A number of neurological disorders have been reported to share a common etiology of defective cholesterol metabolism in the brain [21].

Acetylcholine (ACh) is a neurotransmitter which plays an important role in many functions of both the peripheral and central nervous systems [22]. ACh acts in the processes involved in learning, memory function, locomotor control and cerebral blood flow [23–27]. ACh levels in synaptic cleft are regulated by AChE activity and have been shown to be implicated in cell proliferation [28] and neurite outgrowth [29]. Interestingly, AChE responds to various insults including oxidative stress, an important event that has been related to the pathogenesis and progression of a variety of CNS disorders [30]. Thus, this enzyme may be a target for the emerging therapeutic strategy to treat cognitive disorders caused by hyperlipidemia [31].

Previous experimental investigations have suggested that guaraná (*Paullinia cupana*) may increase metabolism of adipose tissue in rats thereby increasing lipolysis as a result of the activation of adenosine A1 receptors [32] and reduction of body weight [33]. Guaraná is a rich source of caffeine, which may stimulate the metabolism and enhance thermogenesis by acting as an ergogenic agent, assisting in the degradation of lipids and prevention of atherosclerosis [34]. These positive effects may contribute immensely towards the reduction of risk factors for cardiovascular diseases and improve cognitive functions [35, 36]. Leite et al. [37] also reported the presence of high concentration of polyphenols including tannins, flavonoids, and catechins and linked the antioxidant actions to these polypenols [38]. In addition, guaraná may exert several other properties including hypotensive, hypocholesterolemic, and antiinflammatory activities [39].

Since hyperlipidemia is a major risk factor in the development of cognitive dysfunction related memory loss and guarana has been reported for its antioxidant and neuroprotective effects. However, the possible mechanism involved in its neuroprotection has not been fully understood. It is therefore necessary to evaluate the possible preventive therapeutic mechanism of guarana on memory and anxiogenic-like behavior as well as AChE activity and biochemical parameters in hyperlipidemia rat model.

2. Material and methods

2.1. Chemicals

Acetylthiocholine iodide, 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), Coomassie Brilliant Blue G, Caffeine and Poloxamer-407 were obtained from Sigma Chemical Co. (St. Louis, MO,USA). Powdered *P. cupana* seed was produced and supplied by EMBRAPA (Brazilian Corporation of Agricultural Research). The detailed description and determination of the main bioactive compounds present in guaraná used in this study were reported by Bittencourt et al. [40]. The main compounds present in guarana powder (xanthines and catechins) have been analyzed by chromatography (caffeine = 3.754 mg/g, theobromine = 2.065mg/g, total catechins=1.330mg/g, and condensed tannins = 6.747 mg/g). All chemicals used in this experiment had analytical grade and highest purity.

2.2. Animals

Adult male heterogenic Wistar rats (70 - 90 days; 220 - 300 g) from the Central Animal House of the Federal University of Santa Maria (UFSM) were used in this experiment. The animals were maintained at a constant temperature (23 ± 1 °C) on a 12 h light/dark cycle with free access to food and water. All animal procedures were approved by the Animal Ethics Committee (protocol under number: 114/2014).

2.3. Experimental procedure

The rats used in this study were randomly divided into twelve groups that received guarana in doses of 12.5, 25 and 50 mg/kg, caffeine 0.2 mg/kg, human equivalent dose of simvastatin, will serve as a comparative standard, and saline (0.9% NaCl). Group 1; normal rat administered saline (N+S), group 2; normal rat administered 12.5 mg/kg of guaraná (N+12.5), group 3; normal rat administered 25 mg/kg of guaraná (N+25), group 4; normal rat administered 50 mg/kg of guaraná (N+50), group 5; normal rat administered 0.2 mg/kg of caffeine (N+Caf), group 6; normal rat administered simvastatin 0.04 mg/kg (N+Sim), group 7; hyperlipidemic rat administered saline (H+S), group 8; hyperlipidemic rat administered 12.5mg/kg of guarana (H+12.5), group 9; hyperlipidemic rat administered 25 mg/kg of guarana (H+25), group 10; hyperlipidemic rat administered 50 mg/kg of guarana (H+50), group 11; hyperlipidemic rat administered 0.2 mg/kg of caffeine (H+Caf) and group 12; hyperlipidemic rat

administered simvastatin 0.04 mg/kg (H+Sim). Guaraná powder, caffeine and saline were administered by oral gavage once a day for a period of 30 days. The doses of guaraná administered were based on the previous work from our research group [41]. The caffeine concentration chosen was equivalent to the highest dose of guaraná. To ascertain the effects of guaraná powder and caffeine in experimental animal model, the equivalent dose of guaraná powder and caffeine in rats and humans was calculated according to the method described by Reigner and Blesch [42]. Equation 1 was used to convert milligram per kilogram into milligram per square meter to determine animal surface area:

$$BSA\ (m^2) = 1.85\ (W / 70)^{2/3} \quad (1)$$

where BSA is the body surface area and W is the body weight in kilograms. As human, body surface was considered the average value ($1.8m^2$) to a human 70 kg. Substituting into the equation, one rat (0.250kg) was equivalent to one body area $0.0432m^2$. Then converting milligram per kilogram to milligram per square meter. Next, the dose in milligram per square meter was multiplied by human surface area to find the human dose. For doses of 12.5, 25, and 50 mg/kg of guaraná powder, equivalent doses to human of 520.83, 1041.66, and 2083.34mg were obtained, respectively. For dose of 0.2mg/kg of caffeine, equivalent dose to human was 8.33mg.

To induce hyperlipidemia, 500 mg/kg of Poloxamer-407 (dissolved in sterile NaCl 0.9% solution) was administered by a single intraperitoneal injection [17]. The non-hyperlipidemic rat groups received the same volume of vehicle (sterile 0.9% NaCl solution). After induction of hyperlipidemia, the groups destined to receive simvastatin were administered the dose of simvastatin by oral gavage. The simvastatin administered rat groups received only saline before induction. After 36 hours of induction, the animals with higher values of total cholesterol and triglycerides levels when compared to the reference values [16] were subjected to training and behavioral parameter estimation. After the 30 days of experiment, the animals were anesthetized with isoflurane and euthanized, blood was collected by cardiac puncture. The cerebral cortex, hippocampus, striatum, hypothalamus and cerebellum were collected for the subsequent enzymatic assays (Fig.1).

2.4. Separation of blood serum

Animals were anesthetized with isoflurane, and blood was collected through cardiac puncture. Blood samples were collected in tubes without anticoagulant, and after clot formation, they were centrifuged at 3000 rpm for 15 min at room temperature. The resultant serum samples were aliquoted in microtubes and kept on ice for subsequent measurements.

2.5. Biochemical parameters

Serum levels of glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C) triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, creatinine and uric acid were evaluated in a semi-automatic analyzer (TPAnalyzer Plus®, Thermoplate) using commercial kits (Labtest® Diagnóstica S.A.). Tests were carried out in duplicate. The low-density lipoprotein cholesterol (LDL-C) level was calculated using the formula of Friedewald [48].

2.6. Brain tissue preparation

The cranium was opened; the structures were gently removed, separated into the cerebral cortex, hippocampus, striatum, hypothalamus and cerebellum. The brain structures were homogenized in a glass potter in a solution of 10 mM Tris–HCl, with pH7.4, on ice, at a proportion of 1:10 (w/v). The resulting homogenate was centrifuged at 1800 rpm for 10 min and the resulting supernatant was used to determine AChE activity.

2.7. AChE assay

The AChE assay was determined as previously described [46] with a modification of the spectrophotometric method as previously described by Rocha et al. [47]. The reaction mixture (2 ml final volume) contained 100 mM K^+- phosphate buffer, with pH 7.5 and 1 mM 5,5′-dithio-bisnitrobenzoic acid (DTNB). The method is based on the formation of the yellow anion, 5,5′-dithio-bis-acid nitrobenzoic, measured by absorbance at 412 nm during 2 min of incubation at 25 °C. The enzyme was pre-incubated for 2 min. The reaction was initiated by adding 0.8 mM acetylthiocholine iodide (AcSCh). The protein content was adjusted for each structure: cerebral cortex (0.8 mg/ml), hippocampus (0.8 mg/ml), striatum (0.4 mg/ml), hypothalamus (0.6

mg/ml) and cerebellum (0.6 mg/ml). All samples were analyzed in triplicate and the specific activity of the enzyme was expressed in μmol AcSCh/h/mg of protein.

2.8. Protein determination

Protein content was determined colorimetrically according to the method of Bradford [49] using bovine serum albumin (1 mg/ml) as standard solution.

2.9. Behavioral procedure

2.9.1. Object recognition task

After thirty days of treatment with guarana, caffeine or vehicle in rats; the animals were subjected to the object recognition memory task after 12 hours of induction with hyperlipidemia and 24 hours before the euthanasia of the rat. The object recognition memory task was carried out in order to evaluate the short-term memory. An object recognition task was carried out according to the method previously described by Gomes et al. [43]. The task was performed in a 30 × 30 × 30 cm wooden chamber, with walls painted black, a front wall made of Plexiglas and a floor covered with ethyl vinyl acetate sheet. A light bulb, hanging 60 cm above the behavioral apparatus, provided constant illumination of about 40 lux, and an air-conditioner provided constant background sound isolation. The objects used were plastic mounting bricks, each with different shape and colors, but the same size. Throughout the experiments, objects used were arranged in a counterbalanced manner. Animals had not previously displayed a preference for any of the objects. Chambers and objects were cleaned with 30% ethanol immediately before and at the end of each behavioral evaluation. The task consisted of habituation, training, and testing sessions, each lasting 10 minutes. In the first session, rats were individually habituated to the behavioral apparatus and then returned to their home cages. Twenty-four hours later, the animals were subjected to a training session in which the animals were exposed to two of the same objects (object A), and the exploration time was recorded with two stopwatches. Exploration was recorded when the animal touched or reached the object with the nose at a distance of less than 2 cm. Climbing or sitting on the object was not considered exploration. The test session was carried out 4 hours after training. Rats were placed back in the behavioral chamber and one of the familiar objects (object A) was replaced by a novel object (object B). The time spent exploring the familiar and the novel object

was recorded. The discrimination index was then calculated; taking into account the difference in time spent exploring the new and familiar objects, using the Equation 2:

$$[(T_{novel} - T_{familiar}) / (T_{novel} + T_{familiar})] \text{ X } 100(\%) \quad (2)$$

The discrimination index was used as a memory parameter.

2.9.2. Elevated plus maze task

Anxiolytic-like behavior was evaluated using the elevated plus maze task as previously described [44, 45]. The apparatus consisted of a wooden structure raised to 50 cm from the floor. This apparatus was composed of 4 arms of the same size, with two closed-arms (walls of 40 cm) and two open-arms. Initially, the animals were placed on the central platform of the maze in front of an open arm. The animal had 5 min to explore the apparatus. The time spent and the numbers of entries in open and closed arms were recorded. The apparatus was thoroughly cleaned with 30% ethanol between each session.

3. Statistical analysis

The statistical analysis was performed using two-way analysis of variance Newman–Keuls multiple comparison test. $P<0.05$ was considered to represent a significant difference. All data were expressed as mean±standard error of the mean.

4. Results

4.1. Biochemical parameters

The result obtained for the glucose, total cholesterol, LDL-C, HDL-C, triglycerides, AST, ALT, ALP, albumin, creatinine and uric acid levels in both normal and hyperlipidemic rats administered either guaraná, caffeine or simvastatin are shown in Table 1. A significant increase ($P<0.001$; n=7) in total cholesterol was observed in H+S (559%) group when compared to N+S group. However, there were significant reductions ($P<0.05$) in total cholesterol levels of rats in H+25 (17%), H+50 (41%), H+Caf (20%) and H+Sim (47%) groups when compared to H+S group. It is important to note that no significant ($P>0.05$; n=7) change in total cholesterol was observed between H+25 and H+Caf groups as well as between H+50 and H+Sim groups. In addition, significant elevations ($P<0.001$; n=7) in LDL-C were observed in all

hyperlipidemia induced groups: H+S (754%), H+12.5 (609%), H+25 (537%), H+50 (361%), H+Caf (470%) and H+Sim (372%) when compared to N+S group. Similar result pattern was observed for total cholesterol in that no significant difference (P>0.05) in LDL-C was observed between H+25 and H+Caf groups as well as between H+50 and H+Sim groups. A significant increase (P<0.001; n=7) in triglycerides was observed in H+S (1124%) group when compared to N+S group. Furthermore, there were no significant (P>0.001) differences in other biochemical parameters such as glucose, albumin, creatinine and uric acid in serum between hyperlipidemic and normal rat groups.

4.2. Hepatic enzyme activities

The results of the hepatic enzyme activities are presented in Table 2. A significant increase (P<0.001; n=7) in AST activity in H+S (93%) and H+12.5 (60%) groups when compared to N+S group. In addition, the ALT activity was significantly increased in H+S (219.35%) when compared to N+S group (P<0.001; n=7). However, gradual decreases were observed in H+12.5 (5%), H+25 (6%), H+50 (11%), H+Caf (9%) and H+Sim (32%) groups when compared with H+S group. In a similar manner, there was a significant increase in ALP in hyperlipidemic group (124%, P<0.001), except in H+Sim group, when compared to N+S group.

4.3. Behavioral tests

4.3.1. Guarana prevents memory impairment induced by hyperlipidemia.

Fig. 2 shows the preventive effect of guarana (12.5, 25 and 50 mg/kg), caffeine and simvastatin in a rat model of hyperlipidemia subjected to the recognition index test. Statistical analysis (one-way ANOVA) revealed significant difference among the groups in the recognition index test (P<0.001). Post hoc analysis showed that hyperlipidemia significantly (P<0.001) decreased the recognition index. The recognition index decreased from 85.35 ± 5.11 in the N+S group to -62.84 ± 14.94 in the hyperlipidemia group (H+S). H+50 (75.76 ± 4.87) prevented and H+Sim (86.95 ± 5.72) completely reversed the recognition index induced by hyperlipidemia. There was no significant (P>0.05) between the recognition indices between H+50 and H+Sim when compared with N+S group. However, H+12.5 (22.37 ± 9.90), H+25 (38.92 ± 8.59) and H+Caf (34.43 ±7.41) groups, significantly (P<0.05) prevented the hyperlipidemia induced in rats when compared to N+S group. Guarana (12.5, 25, 50

mg/kg), caffeine and simvastatin without hyperlipidemia did not show any significant (P>0.05) effect compared to N+S group (data not shown).

4.3.2 Effect of guarana, caffeine and simvastatin in hyperlipidemic rats on anxiolytic-like behavior.

Fig. 3 shows the effect of guarana, caffeine and simvastatin on the anxiolytic-like behavior in hyperlipidemic rats placed in the elevated plus-maze task. Statistical analysis of testing (one-way ANOVA followed by the Newman–Keuls multiple comparison post-hoc test) showed that the hyperlipidemic rats spent same time in open arms and same number of entries in the open arms when compared to rats of the other groups. This indicates that hyperlipidemia induced with P-407 may not cause anxiogenic effect (Fig. 3A and B). In a similar manner, there was no alteration in the time spent and number of entries in the closed arms by hyperlipidemic rats when compared to the animals in other groups tested. This indicates that hyperlipidemia may not impair locomotor activity in this test (Fig. 3C). Guarana (12.5, 25, 50 mg/kg), caffeine and simvastatin *per se* showed no significant change in the evaluated parameters compared to N+S group (data not shown).

4.4. Guarana prevented the alterations induced by hyperlipemia in AChE activity in the hippocampus.

The results obtained for AChE activity in different brain structures of hyperlipidemic rats treated with guarana, caffeine and simvastatin are presented in Fig. 4. There were no significant (P>0.05) differences in the cerebral cortex, hypothalamus, striatum and cerebellum AChE activities between all the rat groups (Fig. 4A, C, D and E). However, the AChE activity was significantly (P<0.05) decreased (60%) in the hippocampus of H+S group when compared to N+S group. However, pretreatment with 12.5, 25 and 50 mg/kg of guarana, caffeine and the treatment with simvastatin significantly (P<0.05) increased AChE activity when compared to H+S group (P<0.05). Only guarana 50 mg/kg and simvastatin groups were able to significantly (P<0.01) prevented and reversed respectively, the decreased AChE activity in hyperlipidemia. It is worth noting that the treatment with caffeine and 50 mg/kg of guarana had the same effect on hyperlipidemic groups (Fig. 4B). As observed, AChE activity was not modified by guarana, caffeine and simvastatin *per se* treatment in all the brain structures.

5. Discussion

Hyperlipidemia is a common metabolic disorder characterized by diverse alteration in lipid profiles such as hypercholesterolemia and hypertriglyceridemia leading to atherosclerosis, obesity and cardiovascular disease [2]. It may however induce adverse effects in several animal and human brain disorders, leading to memory loss and cognitive dysfunction [3, 50]. In this study, we evaluate the possible preventive effect of guarana in memory and anxiogenic-like behavior as well as AChE activity in hyperlipidemia induced rats. The observed elevations in TC, LDL-C and triglycerides levels in hyperlipidemic group in this study, confirm the efficacy of induction. Similar findings were reported earlier by Johnston (2004) [15] following an identical single dose of poloxamer 407 (P-407). It has been used to induce experimental hyperlipidemia in several rodent species including rat [16], mouse [17] and rabbit [51]. It is worth noting that P-407 has the ability to cross the BBB, hence its adverse effect on the structure and function of the brain has been well documented [13, 14]. The possible mechanisms by P-407 induce hypertriglyceridemia and hypercholesterolemia may include the inhibition of lipoprotein lipase, stimulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity and reduction of low density lipoprotein (LDLr) receptor expression in all synthesizing cholesterol cells [15-17].

As observed in this study, pretreatment with guarana powder and caffeine as well as treatment with simvastatin in hyperlipidemic states, showed significant reductions in TC and LDL-C levels when compared to the hyperlipidemic group without pretreatment (H+S). This may indicate possible preventive effect of guarana powder and caffeine against the alterations caused by hyperlipidemia. This result corroborates with data from the literature where cholesterol was lowered and LDL-C levels reduced by guarana powder in humans [39]. In this experiment, both the caffeine as simvastatin were used as standard compound and drug respectively. Their effects were subsequently compared with guarana powder. Caffeine at the dose tested (0.2 mg/kg) was also able to reduce the levels of cholesterol and LDL-C but not in the same proportion as 50 mg/kg of guarana. This result corroborates with data obtained from a study where hypercholesterolemic rats treated with caffeine, were reported to show decreased levels of LDL-C and total cholesterol levels [53]. The difference in the effect of the same concentration of caffeine and guarana, was documented in our previous investigation [41]. In a similar manner, simvastatin (lipophilic statin drug) used as a

standard in this study, was able to lower cholesterol and LDL levels of hyperlipidemic rats. The reduction by simvastatin was similar to that of the highest dose of guarana, when compared to the hyperlipidemic group. Indeed, simvastatin is an inactive prodrug that is hydrolyzed to its active form in liver and plasma. Although, the active moieties of all statins may have low blood-brain barrier permeability; however, the hydrolyzed simvastatin may in turn enter the brain through carrier-mediated transport and selectively acts by inhibiting HMG-CoA reductase in cholesterol biosynthesis in the liver and in the brain [54].

Concerning hepatic enzymes, the increased serum ALT, AST and ALP activities in hyperlipidemic groups are in agreement with findings from the work reported of Sheneni et al. [52], which indicated that disorders in lipoprotein metabolism may potentially be accompanied by hepatic injury. The accumulation of triglycerides and other fats in the liver cells may thus contribute to elevations in serum ALT, AST and ALP activity in P-407 treated rats [52]. In relation to the hepatic enzymes, we found increased ALP activity in all hyperlipidemic groups, except in H+Sim. ALT activity was increased in all of hypercholesterolemic group. However, H+Sim group presented a slight decrease of this hepatic enzyme when compared to H+S group. AST activity was altered only in H+S and H+12.5 groups. Aminotransferases and ALP are normally found in circulation in small amounts because of hepatic growth and repair. In clinical examination, the increased levels of these enzyme activities in serum are well known biomarkers for liver damage. Nevertheless, approximately 80% of AST in hepatocytes appears to be located in the mitochondria, whereas ALT is thought to be predominantly non-mitochondrial. ALT has been implicated in mild hepatocellular injury, in which the hepatocytes are plasmatic but the mitochondrial membrane is not damaged [55].

Our results have revealed that P-407 induced hyperlipidemia can damage both the plasmatic and mitochondrial membrane. However, pretreatment with guarana and caffeine, as well as treatment with simvastatin may prevent and/ or reduce mitochondrial level damage; possibly due to reduction in cholesterol accumulation in the liver cells [56].

The novel object recognition task has been used for evaluating memory function in rodents [43]. As observed in this study, the significant decrease in the recognition index of hyperlipidemic rats may suggest possible impairment of short-term memory function. This result corroborates with published data where hyperlipidemia induced by intraperitoneal administration of poloxamer resulted into cognitive impairment [57].

Increased permeability of the BBB has also been observed in rats and rabbits with increased circulating cholesterol [58 - 60]. Other studies have linked high cholesterol to damage of the BBB with corresponding impairments in hippocampal-dependent memory tasks [61]. However, pretreatment of hyperlipidemic rats with highest dose of guarana (H+50 group) in this study, resulted into the recognition index that was similar to that of the N+S group. These findings indicate that treatment with guarana may prevent memory impairment induced by hyperlipidemia. This agrees with previous studies where guarana has been reported to prevent memory loss and improve cognition in rats [62] and humans [63, 64]. Furthermore, treatment of hyperlipidemic rats with simvastatin (H+Sim) also resulted into the recognition index similar to the N+S group. This result corroborates with previous reporter hyperlipidemic mice were treated with simvastatin and pravastatin, showed improvement in memory and learning functions [65].

In the novel object recognition task, hyperlipidemic rats pretreated with caffeine (H+Caf), and guarana (H+50), showed significant elevations in recognition indices when compared with the H+S group. This agrees with earlier study where improved memory function was reported when low doses of caffeine (0.2 to 20 mg/kg) were administered to rats and mice [66]. The short-term memory improvement of guarana may not be completely linked to caffeine; possibly other components of guarana may act either in additive manner. These results follow similar trend with the report of Espinola et al. [62] where guarana was shown to have a better performance than the same concentration of caffeine *per se* in the memory of mice by reversing the amnesic effect of scopolamine.

Caffeine has psychostimulant effects on the central nervous system (CNS). The blockade of adenosine A_1 and A_{2A} receptors is considered the primary pharmacological target of caffeine and this non-selective antagonism seems to be responsible for the psychostimulant effect of caffeine in the CNS [67]. Adenosine A_1 receptors are expressed densely in the cerebral cortex and hippocampus. Activation of adenosine A_1 receptors strongly inhibits the release of acetylcholine from pyramidal hippocampal neurons. Acetylcholine has been shown to be important for memory storage [68].

After the session of novel object recognition, animals were subjected to elevated plus-maze task and anxiogenic-like behavior was evaluated. Our results showed that the hyperlipidemia induced with P-407 did not significantly alter the time spent in open arms, number of entries in open arms and number of entries in closed arms between the

rat groups. This suggests that neither hyperlipidemic rat groups nor the groups pretreated with guarana, caffeine nor treated with simvastatin *per se* was anxious; indicating that no anxiogenic-like behavior was observed in all the groups studied. This is in agreement with the assertion with a study on hypercholesterolemia where no significant difference was found among the groups in the elevated plus maze test, suggesting that elevated serum levels of cholesterol may not cause anxiogenic-like behavior [69].

The effect of hyperlipidemia on the cholinergic system is another important aspect that was considered in this study. The cholinergic system plays a crucial role in regulating learning, memory, and cortical organization of movement [70]. Acetylcholinesterase (AChE) is a pivotal enzyme which hydrolyzes the neurotransmitter acetylcholine (Ach) in many tissues and responsible for modulating the cholinergic function [28]. In the present study, there was significant decrease in AChE activity in the hippocampus of hyperlipidemic rat group when compared with the normal control group. However, there were no significant differences in the AChE activity in the other brain structures including cerebral cortex, hypothalamus, striatum and cerebellum between all the groups. The results obtained agree in part, with that obtained by Gutierres et. al. [71] where diet induced hypercholesterolemic rats had significantly reduced AChE activities in the synaptosomes of cerebral cortex and hippocampus. The non significant difference in the AChE activity in cerebral cortex in this study may be attributed to the short exposure time (acute exposure) to P-407 for the development of hyperlipidemia (36 hours) unlike the hypercholesterolemia induced by diet which took a longer period of 4 weeks to develop [72]. This shows that the hippocampus, due to a higher cholesterol concentration than other brain structures, responds more quickly to hyperlipidemia than other brain structures [58]. Changes in hippocampus-specific memory are seen in hyperlipidemia, as the effects on non-dependent memory tests hippocampus, showed no changes [73, 74].

Cholesterol plays extensive role in cell membrane fluidization and facilitates transmembrane signaling by altering membrane function. These effects have been attributed to bulk lipid loading of the membrane, by direct interaction of cholesterol with the cell membrane resulting in disruption of the ability of the protein to undergo the conformational changes required for signaling [75]. Cholesterol is synthesized *in situ* in the brain and found in a small amount of plasma. The renewal of brain cholesterol may occur by conversion of cholesterol in oxysterols. This compound that

can cross the BBB and promote protein infiltration, a process that may contribute to the development of Alzheimer's disease [12].

In this regard, alterations in membrane lipid and cholesterol contents have been reported to alter the activities of intrinsic membrane enzymes [56]. Specifically, AChE is anchored onto the cell membranes by the transmembrane protein; proline-rich membrane anchor (PRiMA) as a tetrameric globular form that is prominently expressed in vertebrate brain. Interestingly, there is evidence that part of the PRiMA-linked AChE may be integrated in membrane lipid rafts, regions with a high concentration of cholesterol that could modulate enzyme activity [76]. It is important to note that reduction in AChE activity, caused by hyperlipidemia can lead to a lesser hydrolysis of ACh neurotransmitter in synapse and consequently an abnormal amount of this neurotransmitter can cause an overactivation of cholinergic receptors and elicit possible toxic effects [77]. On the other hand, it has been reported that the AChE activation leads to fast ACh degradation and a subsequent down stimulation of ACh receptors causing undesirable effects on cognitive functions [78]. In the present study, hyperlipidemic rats showed a decrease in the AChE activity, and reduction of memory function. These results corroborate with literature in that rats fed a hypercholesterolemic diet showed a decrease in choline acyltransferase in ChAT-positive neurons [79], thereby leading to reduction in acetylcholine production in hippocampus. Thus, the reduction of acetylcholine level may be linked to the decreased cognitive ability. Pretreatment with guarana and the treatment with simvastatin in hyperlipidemic rats, may have led to the reduction in ChAT and subsequent normalization of acetylcholine levels by modulating AChE activity in hippocampus to that similar to control group. The results from this study agree with previous studies where guarana was reported to prevent memory loss and improve cognitive function in rats [58] and humans [59, 60]. Furthermore, the compound (caffeine), drug (simvastatin) and guarana modulated ACh level in the brain structures directly or indirectly in hyperlipidemic rat. In an attempt to confer neuroprotection, simvastatin may inhibit HMG-CoA reductase in cholesterol biosynthesis in the liver and in the brain [62], alter neurotransmitter level, elevate AChE activity and improve memory and learning [61].

In conclusion, our data revealed that guarana powder was able to reduce TC and LDL-C levels, in a similar manner to simvastatin and protect the liver against damage caused by hyperlipidemia. Furthermore, induction of hyperlipidemia by P-407 did not present anxiolytic-like behavior in rats. However, guarana powder was able to prevent

the alterations of AChE activity in the hippocampus and ameliorated memory impairment induced by hyperlipidemia. There is significant difference in the modulatory effects of caffeine (same concentration present in the highest dose of guarana) and guarana, bringing out the scientific prominence of this work. Although, further study is required to validate the neuroprotective effect of guarana in hyperlipidemic condition, guarana powder may be a source of promising phytochemicals that can be used as an adjuvant therapy in the management of hyperlipidemia and cognitive disorders.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

References

[1] Veghari G, Sedaghat M, Maghsodlo S et al. The association between abdominal obesity and serum cholesterol level. Int J App Basic Med Res 2015;5(2):83–86.

[2] De Winter CF, Bastiaanse LP, Hilgenkamp TIM, Evenhuis HM, Echteld MA. Cardiovascular risk factors (diabetes, hypertension, hypercholesterolemia and metabolic syndrome) in older people with intellectual disability: results of the HA-ID study. Res Dev Disabil 2012;33(6):1722–1731.

[3] Dias IHK, Polidori MC, Griffiths HR. Hypercholesterolaemia-induced oxidative stress at the blood-brain barrier. Biochem Soc Trans 2014;42(4):1001–1005.

[4] Goritz C, Mauch DH, Nagler K, Pfrieger FW. Role of glia-derived cholesterol in synaptogenesis: new revelations in the synapse–glia affair. J Physiol Paris 2002;96:257–263.

[5] Koudinov AR, Berezov TT. Cholesterol, statins, and Alzheimer disease. PLoS Med 2005;2:E81.

[6] Mauch DH, Nagler K, Schumacher S, et al. CNS synaptogenesis promoted by glia-derived cholesterol. Science 2001;294:1354–1357.

[7] Klopfenstein DR, Tomishige M, Stuurman N, Vale RD. Role of phosphatidylinositol (4,5)bisphosphate organization in membrane transport by the Unc104 kinesin motor. Cell 2002;109:347– 358.

[8] Dietschy JM, Turley SD. Cholesterol metabolism in the brain. Curr Opin Lipidol 2001;12:105–112.

[9] Dietschy JM, Turley SD. Thematic review series: brain lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. J Lipid Res 2004;45:1375–1397.

[10] Shobab LA, Hsiung GY, Feldman HH. Cholesterol in Alzheimer's disease. Lancet Neurol 2005;4:841–852.

[11] Simons M, Keller P, Dichgans J, Schulz JB. Cholesterol and Alzheimer's disease: is there a link? Neurol 2001;57(6):1089-93.

[12] Locatelli S, Lutjohann D, Schmidt HH, Otto C, Beisiegel U, Von Bergmann K. Reduction of plasma 24S-hydroxycholesterol (cerebrosterol) levels using highdosage simvastatin in patients with hypercholesterolemia. Neurol 2002;59(2):213-216.

[13] Kulkarni SA, Feng SS. Effects of surface modification on delivery efficiency of biodegradable nanoparticles across the blood-brain barrier. Nanomedicine 2011;6(2):377-394.

[14] Petri B, Bootz A, Khalansky A, et al. Chemotherapy of brain tumour using doxorubicin bound to surfactant-coated poly(butyl cyanoacrylate) nanoparticles: revisiting the role of surfactants. J Control Release. 2007;117:51-58.

[15] Johnston TP. *(*2004*).* The P-407-induced murine model of dose-controlled hyperlipidemia and atherosclerosis: A review of findings to date. J Cardiovasc Pharmacol 2004;43:595–606.

[16] Lee YS, Kim YW, Kim SG, et al. Effects of poloxamer 407-induced hyperlipidemia on the pharmacokinetics of carbamazepine and its 10, 11-epoxide metabolite in rats: Impact of decreased expression of both CYP3A1/2 and microsomal epoxide hydrolase. Eur Neuropsychopharmacol 2012;22(6):431-440.

[17] Palmer WK, Emeson EE, Johnston TP. Poloxamer 407-induced atherogenesis in the C57BL/6 mouse. Atherosclerosis 1998;136(1):115-123.

[18] Simons K, Ikonen E. Functional rafts in cell membranes. Nature 1997;387:569-572.

[19] Lasserre R, Guo XJ, Conchonaud F, Hamon Y, Hawchar O, et al. Raft nanodomains contribute to Akt/PKB plasma membrane recruitment and activation. Nat Chem Biol 2008;4:538-547.

[20] Papakostas GI, Öngür D, Iosifescu DV, Mischoulon D, Fava M. Cholesterol in mood and anxiety disorders: review of the literature and new hypotheses. Eur Neuropsychopharmacol 2004;14:135-142.

[21] Porter FD, Herman GE. Malformation syndromes caused by disorders of cholesterol synthesis. J Lipid Res 2011;52:6-34.

[22] Scarr E. Muscarinic receptors: their roles in disorders of the central nervous system and potential as therapeutic targets. CNS Neurosci Ther 2012;18(5):369-379.

[23] Kiehn O. Locomotor circuits in the mammalian spinal cord. Annu Rev Neurosci 2006;29:279-306.

[24] Deiana S, Platt B, Riedel G. The cholinergic system and spatial learning. Behav Brain Res 2011;221:389-411.

[25] Hut RA, Van der Zee EA. The cholinergic system, circadian rhythmicity, and timememory. Behav Brain Res 2011;221:466-80.

[26] Klinkenberg I, Sambeth A, Blokland A. Acetylcholine and attention. Behav Brain Res 2011;221:430-42.

[27] Havekes R, Abel T, Van der Zee EA. The cholinergic system and neostriatal memory functions. Behav Brain Res 2011;221:412-23.

[28] Appleyard ME. Non-cholinergic functions of acetylcholinesterase. Biochem Soc Trans 1994;22:749-55.

[29] Chacon MA, Reyes AE, Inestrosa NC. Acetylcholinesterase induces neuronal cell loss, astrocyte hypertrophy and behavioral deficits in mammalian hippocampus. J Neurochem 2003;87:195–204.

[30] Ozkul A, Akyol A, Yenisey C, Arpaci E, Kiylioglu N, Tataroglu C. Oxidative stress in acute ischemic stroke. J Clin Neurosci 2007;14:1062–6.

[31] Hegazy AM, et al. Comparative study of cholinergic and oxidative stress biomarkers in brains of diabetic and hypercholesterolemic rats. Hum Exp Toxicol 2015:0960327115583361.

[32] Lima WP, Carnevali LC Jr, Eder R, Costa Rosa LF, Bacchi EM, Seelaender MC. Lipid metabolism in trained rats: effect of guarana (Paullinia cupana Mart.) supplementation. Clin Nutr 2005;24:1019-1028.

[33] Boozer CN, Nasser JA, Heymsfield SB, Wang V, Chen G, Solomon JL. An herbal supplement containing Ma Huang-Guarana for weight loss: a randomized, double-blind trial. Int J Obes Relat Metab Disord 2001;25:316-324.

[34] Shimada K, Kawarabayashi T, Tanaka A, et al. Oolong tea increases plasma adiponectin levels and low-density lipoprotein particle size in patients with coronary artery disease. Diabetes Res Clin Pract 2004;65:227-234.

[35] Haskell CF, Kennedy DO, Wesnes KA, Milne AL, Scholey AB. A double-blind, placebo-controlled, multi-dose evaluation of the acute behavioural effects of guaraná in humans. Psychopharmacol 2007;21(1):65-70.

[36] Kennedy DO, et al. Improved cognitive performance and mental fatigue following a multi-vitamin and mineral supplement with added guaraná (Paullinia cupana). Appetite 2008;50(2-3):506-13.

[37] Leite RP, Wada RS, Monteiro JC, Predes FS, Dolder H. Protective effect of guaraná (Paullinia cupana var. sorbilis) pre-treatment on cadmium-induced damages in adult wistar testis. Biol Trace Elem Res 2011;141(1-3):262-274.

[38] Razzaghi-Asl N, Garrido J, Khazraei H, Borges F, Firuzi O. Antioxidant properties of hydroxycinnamic acids: a review of structure-activity relationships. Curr Med Chem 2013;20(36):4436-4450.

[39] Krewer CC, Ribeiro EE, Ribeiro EA, et al. Habitual intake of guaraná and metabolic morbidities: an epidemiological study of an elderly Amazonian population. Phytother Res 2011;25:1367-1374.

[40] Bittencourt LS, Machado DC, Machado MM, et al. The protective effects of guaraná extract (Paullinia cupana) on fibroblast NIH-3 T3 cells exposed to sodium nitroprusside. Food Chem Toxicol 2013;53:119-125.

[41] Ruchel JB, et al. Hypercholesterolemia and Ecto-enzymes of Purinergic System: Effects of Paullinia cupana. Phytother Res 2015;30(1):49-57.

[42] Reigner BG, Blesch K. Estimating the starting dose for entry into humans: principles and practice. Eur J Clin Pharmacol 2002;57(12):835-845.

[43] Gomes GM, Dalmolin GD, Bar J, Karpova A, Mello CF, Kreutz MR, et al. Inhibition of the polyamine system counteracts β-amyloid peptide-induced memory impairment in mice: involvement of extrasynaptic NMDA receptors. PLoS One. 2014;9:e99184.

[44] Frussa-Filho R, Barbosa-Junior H, Silva RH, Da Cunha C, Mello CF. Naltrexone potentiates the anxiolytic effects of chlordiazepoxide in rats exposed to novel environments. Psychopharmacol (Berl) 1999;147:168–73.

[45] Rubin MA, Albach CA, Berlese DB, Bonacorso HG, Bittencourt SR, Queiroz CM, et al. Anxiolytic-like effects of 4-phenyl-2-trichloromethyl-3H-1,5-benzodiazepine hydrogen sulfate in mice. Braz J Med Biol Res 2000;33:1069-73.

[46] Ellman GL, Courtney KD, Andres V, Feather-Stone Jr RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88–95.

[47] Rocha JB, Emanuelli T, Pereira ME. Effects of early undernutrition on kinetic parameters of brain acetylcholinesterase from adult rats. Acta Neurobiol Exp (Wars) 1993;53:431–7.

[48] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

[49] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.

[50] Eskelinen MH, Ngandu T, Helkala EL, Tuomilehto J, Nissinen A, Soininen H et al. Fat intake at midlife and cognitive impairment later in life: a population-based CAIDE study. Int J Geriatr Psychiatry 2008;23(7):741-747

[51] Blonder JM, Baird L, Fulfs JC, Rosenthal GJ. Dose-dependent hyperlipidemia in rabbits following administration of poloxamer 407 gel. Life Sci 1999; 65: PL261-266.

[52] Sheneni VD, James DB, Atawodi DE. Ethanolic Extracts of Vitex Doniana Possesses Hepatocuractive Property in Poloxamer Induced Hyperlipidemia, Science Res 2014;2(3):49-54.

[53] Xu Y, Zhang M, Wu T, Dai S, Xu J, Zhou Z. The anti-obesity effect of green tea polysaccharides, polyphenols and caffeine in rats fed with a high-fat diet. Food Funct 2015;6(1): 296–303.

[54] Serrano-Pozo A, et al. Effects of simvastatin on cholesterol metabolism and Alzheimer disease biomarkers. Alzheimer Dis Assoc Disord 2010;24(3):220.

[55] Jiang ZG, Mukamal K, Tapper E, Robson SC, Tsugawa Y. Low LDL-C and high HDL-C levels Are associated with elevated serum transaminases amongst adults in the United States: a cross-sectional study. PLoS One 2014;9(1): e85366.

[56] Ktari N, et al. Cholesterol regulatory effects and antioxidant activities of protein hydrolysates from zebra blenny (Salaria basilisca) in cholesterol-fed rats. Food Funct 2015;6(7):2273-2282.

[57] Clarke MSF, Prendergast MA, Terry AV. Plasma membrane ordering agent pluronic F-68 (PF-68) reduces neurotransmitter uptake and release and produces learning and memory deficits in rats. Learning & memory 1999;6(6):634-649.

[58] Gan L, et al. A 72-hour high fat diet increases transcript levels of the neuropeptide galanin in the dorsal hippocampus of the rat. BMC neuroscience 2015;16(1):1.

[59] Sparks DL, Kuo YM, Roher A, Martin T, Lukas RJ. Alterations of Alzheimer's disease in the cholesterol-fed rabbit, including vascular inflammation. Ann NY Acad Sci 2000;903:335-344.

[60] Ghribi O, Golovko MY, Larsen B, Schrag M, Murphy EJ. Deposition of iron and beta-amyloid plaques is associated with cortical cellular damage in rabbits fed with long-term cholesterol-enriched diets. J Neurochem 2006;99(2):438-449.

[61] Kanoski SE, Zhang Y, Zheng W, Davidson TL. The effects of a highenergy diet on hippocampal function and blood-brain barrier integrity in the rat. J Alzheimer's Dis 2010;21(1):207-219.

[62] Espinola EB, et al. Pharmacological activity of Guarana (Paullinia cupana Mart.) in laboratory animals. J Ethnopharmacol 1997;55(3):223-229.

[63] Veasey RC, et al. The Effects of Supplementation with a Vitamin and Mineral Complex with Guaraná Prior to Fasted Exercise on Affect, Exertion, Cognitive Performance, and Substrate Metabolism: A Randomized Controlled Trial. Nutrients 2015;7(8):6109-6127.

[64] Scholey A, et al. Acute effects of different multivitamin mineral preparations with and without Guaraná on mood, cognitive performance and functional brain activation. Nutrients 2013;5(9):3589-3604.

[65] Ghodke RM, Tour N, Devi K. Effects of statins and cholesterol on memory functions in mice. Metab brain dis 2012;27(4):443-451.

[66] Angelucci MEM, et al. Effects of caffeine on learning and memory in rats tested in the Morris water maze. Braz J Med Biol Res 2002;35(10):1201-1208.

[67] Ferré S. An update on the mechanisms of the psychostimulant effects of caffeine. J Neurochem 2008;105:1067-79

[68] Everitt BJ, Robbins TW. Central cholinergic system and cognition. Annu Rev Psychol 1997;48:649-684.

[69] Pontes MC, Heck LC, Coelho JC. Behavioral and biochemical effects of pharmacopuncture (ST 36 and ST 25) in obese rats. BMC Complement Alter Med 2015;15(1):297.

[70] Mesulam MM, Guillozet A, Shaw P, Levey A, Duysen EG, Lockridge O. Acetylcholinesterase knockouts establish central cholinergic pathways and can use butyrylcholinesterase to hydrolyze acetylcholine. Neurosci Res 2002;110:627–39.

[71] Gutierres JM, et al. Protective effect of α-Tocopherol on memory deficits and Na+, K+-ATPase and acetylcholinesterase activities in rats with diet-induced hypercholesterolemia. Biomedicine & Aging Pathology 2012;2(3):73-80.

[72] Otunola GA, et al. Effects of diet-induced hypercholesterolemia on the lipid profile and some enzyme activities in female Wistar rats. Afr J Biochem Res 2010;4(6):149-154.

[73] Beilharz JE, Maniam J, Morris MJ. Short exposure to a diet rich in both fat and sugar or sugar alone impairs place, but not object recognition memory in rats. Brain Behav Immun 2014;37:134-141.

[74] Kanoski SE, Davidson TL. Different patterns of memory impairments accompany short- and longer-term maintenance on a highenergy diet. J Exp Psychol Anim Behav Process 2010;36(2):313-319.

[75] Devi DR, Sandhya P, Hari BNV. Poloxamer: A novel functional molecule for drug delivery and gene therapy. J Pharm Sci Res 2013;5:159-165.

[76] Xie HQ, Leung KW, Chen VP, Chan GK, Xu SL, Guo AJ, Zhu KY, Zheng KY, Bi CW, Zhan JY, Chan WK, Choi RC, Tsim KW. PRiMA directs a restricted localization of tetrameric AChE at synapses. Chem Biol Interact 2010;187:78-83.

[77] Tougu V, Kesvatera T. Role of ionic interactions in cholinesterase catalysis. Biochim Biophys Acta 1996;1298:12–30.

[78] Soreq H, Seidman S. Acetylcholinesterase-new roles for an old actor. Nat Rev Neurosci 2001;2:294–302.

[79] Ullrich C, Pirchl M, Humpel C. Hypercholesterolemia in rats impairs the cholinergic system and leads to memory deficits. Mol Cell Neurosci 2010;45(4):408-417.

Figure captions

Fig. 1 Experimental Procedure Design *in vivo* (Elaborated by the authors)

Fig. 2 Guarana prevents the impairment of memory induced by hypercholesterolemia. Rats received guarana 12.5, 25 and 50 mg/kg, caffeine (0.2 mg/kg), simvastatin (0,04 mg/kg) and saline (S) by gavage once a day, 7 days a week, for a period of 1 month, except simvastatin, which was administered by gavage after the induction of hyperlipidemia by 500 mg/kg of Poloxamer-407 (i.p.) Four hours after training, the animals were subjected to the novel object recognition test session. H+50 (hyperlipidemia+guarana 50mg/kg) and H+Sim (hyperlipidemia + simvastatin) groups reversed the impairment of memory induced by hyperlipidemia. Data are expressed as mean ± Standard Error of the Mean (SEM). *P<0.05 compared with N+S group (one-way ANOVA followed by the Newman–Keuls multiple comparison post-hoc test).

Fig. 3 Effect of guarana 12.5, 25 and 50 mg/kg, caffeine0.2 mg/kg(Caf) and simvastatin0,04mg/kg(Sim) in hyperlipidemic rats on time in open arms (A), number of entries in open arms (B), number of entries in closed arms (C) measured in the elevated plus maze over the 5 min test. S represent of saline solution administration. Data are expressed as mean ± Standard Error of the Mean (SEM). P>0.05 compared with N+S group (one-way ANOVA followed by the Newman–Keuls multiple comparison post-hoc test).

Fig. 4 Acetylcholinesterase (AChE) activity in cerebral cortex (A), hippocampus (B),hypothalamus (C), striatum(D) and cerebellum(E) of rats preventively treated with guarana (12.5, 25 and 50 mg/kg), caffeine (Caf) and simvastatin (Sim) with hyperlipidemia induced. S represent of saline solution administration.The results were analyzed using two-way ANOVA-Newman-Keuls multiple comparison test and expressed as mean ± S.E.M.*The value is significantly different from N+S group(P< 0.05, n = 7).$^{\#}$The value is significantly different from H+S group(P< 0.05, n = 7).

Fig.1

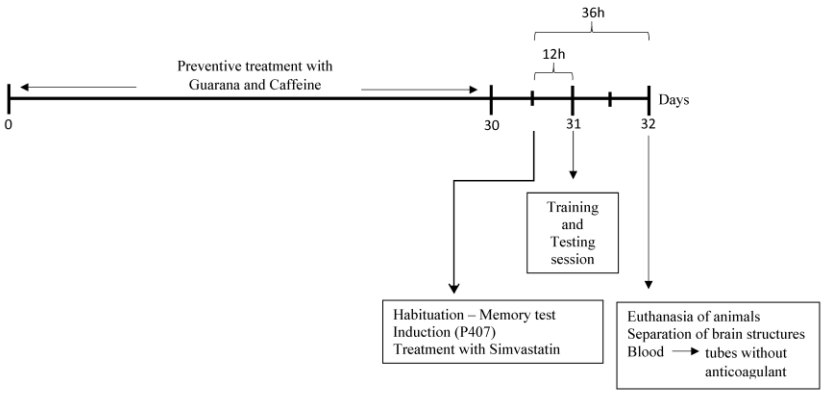


Fig. 2

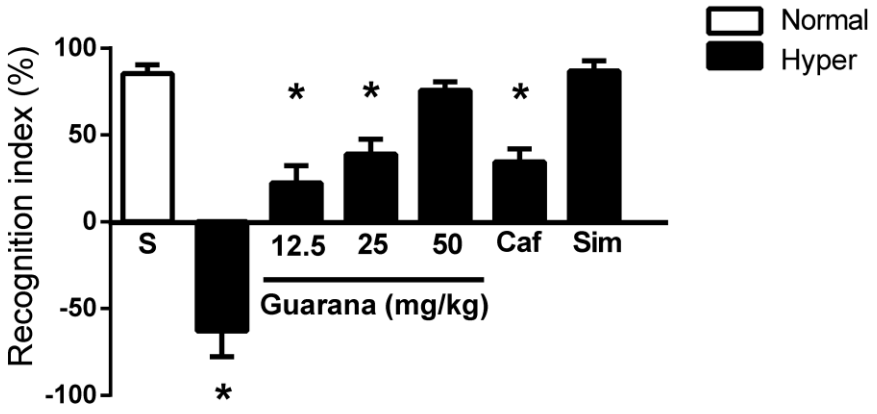


Fig. 3

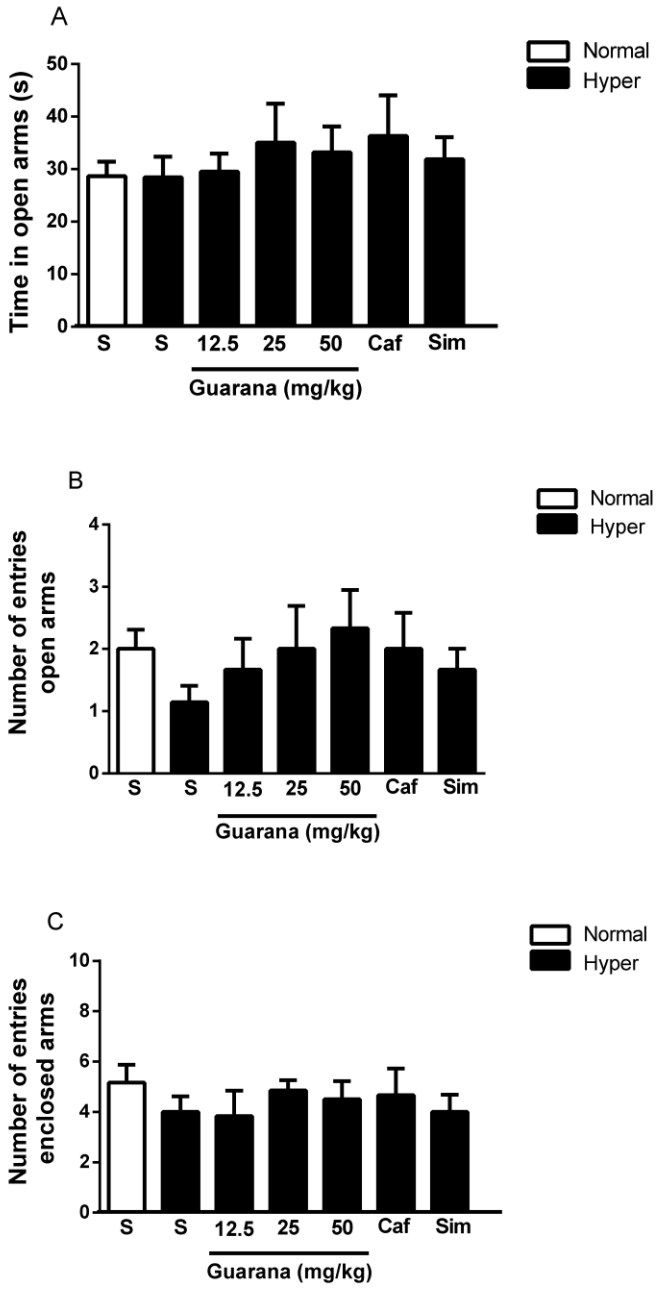
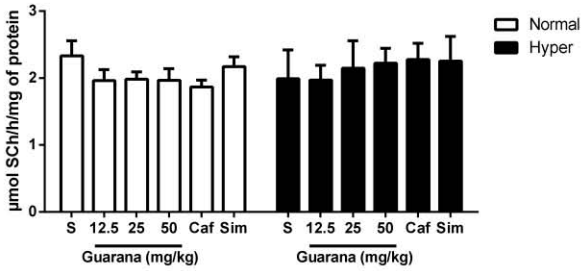


Fig. 4

A



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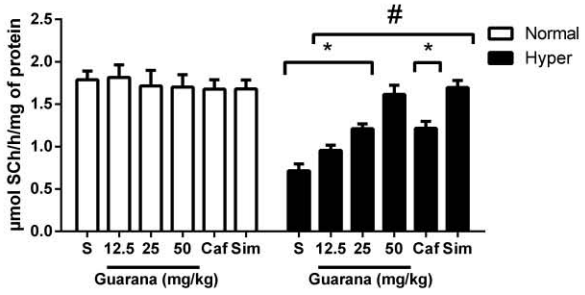

C

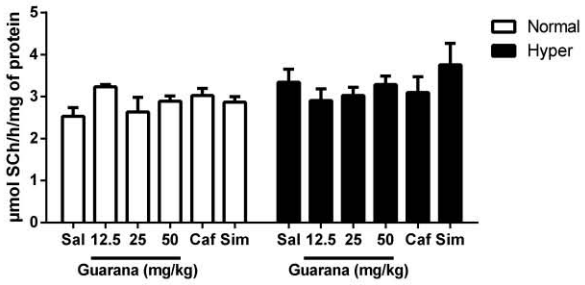

Table 1. Biochemical parameters of the animals hyperlipidemia induced or not and previously treated with guaraná, or caffeine and treated with simvastatin.

Groups	Glucose	TC	LDL-C	HDL-C	Tri	Albumin	Creatinine	Uric Acid
N+S	124.8 ± 3.31	145.6± 9.90	52.66± 3.92	61.07± 6.56	132.2± 21.11	4.01± 0.20	0.51± 0.02	2.76± 048
N+12.5	130.8 ± 18.53	168.6±13.71	48.99± 3.27	57.37± 4.77	145.0± 15.50	4.48± 0.12	0.53± 0.02	2.50± 0.44
N+25	122.4 ± 9.62	164.8± 12.26	51.00± 2.51	51.77± 4.35	155.0± 29.11	4.40± 0.13	0.51± 0.03	2.87± 0.38
N+50	119.3 ± 4.14	142.8±11.65	49.37± 4.50	61.08± 8.51	161.8± 11.73	4.33± 0.07	0.45± 0.02	2.75± 0.36
N+Caf	125.7 ± 6.35	138.3± 11.23	53.62± 3.44	58.40± 4.93	170.3± 16.37	4.50± 0.14	0.51± 0.04	2.02± 0.21
N+Sim	132.7 ± 13.3	131.1± 7.50	49.49± 4.27	53.13± 4.68	163.9± 18.05	3.97± 0.15	0.46± 0.03	2.10± 0.23
H+S	134.5 ± 8.55	960± 133.8***	449.8± 127.6***	46.34± 1.36	1619±209.2***	4.48± 0.15	0.43± 0.09	3.32± 0.32
H+12.5	126.1 ± 6.79	932.0± 87.49***	373.3± 117.6***	47.97± 2.42	1595± 154.0***	4.56± 0.29	0.40± 0.09	3.22± 0.38
H+25	129.9 ± 7.58	797.7± 61.27**	335.4± 56.71**	56.40± 5.36	1214± 227.0***	4.35± 0.10	0.34± 0.08	2.30± 0.29
H+50	121.72 ±4.55	562.8± 65.83*	242.9± 21.67*	68.95± 5.05	1342± 64.25***	4.80± 0.13	0.38± 0.07	2.68± 0.23
H+Caf	127.4 ± 4.12	767.6± 71.97**	300.1± 11.67**	58.98± 7.28	1282± 131.5***	4.52± 0.16	0.37± 0.11	3.32± 0.38
H+Sim	123.0± 4.74	509.7± 67.37*	248.5± 11.40*	70.59± 6.40	1174± 156.4***	4.83± 0.33	0.48± 0.12	2.58± 0.38

Glucose (mg/ dL); TC: total cholesterol (mg/dL); LDL-C: low-density lipoprotein-cholesterol (mg/dL) HDL-C: high-density lipoprotein-cholesterol (mg/dL); Tri: triglycerides (mg/dL); Albumin (g/dL); Creatinine (mg/dL); Uric Acid (mg/dL) . The results were analyzed using two-way ANOVA-Newman-Keuls multiple comparison test and expressed as mean ± S.E.M.*The value is significantly different from normal rats plus saline group ($P< 0.05$, n = 7). **The value is significantly different from normal rats plus saline group ($P< 0.01$, n = 7) and ***The value is significantly different from normal rats plus saline group ($P< 0.001$, n = 7).

Table 2. Hepatic enzymes activities of the animals hyperlipidemia induced or not and previously treated with guaraná, or caffeine and treated with simvastatin.

Groups	AST	ALT	ALP
N+S	215.3± 21.35	47.69± 2.38	425.3± 61.24
N+12.5	150.0± 4.24	56.90± 1.34	621.6± 46.27
N+25	173.6± 11.57	56.00± 5.63	593.5± 124.8
N+50	173.8±17.40	55.50± 4.96	478.8± 44.70
N+Caf	179.6± 13.80	53.38± 1.65	617.6± 57.33
N+Sim	189.0± 16.27	46.50± 4.02	454.8± 56.93
H+S	416.6± 28.36***	152.3± 12.60***	952.3± 106.5***
H+12.5	345.4± 28.36***	143.0± 11.70***	812.4± 73.34***
H+25	292.7± 29.27	144.7± 11.06***	792.0± 48.25***
H+50	229.5± 34.29	135.8± 10.70***	792.0± 61.77***
H+Caf	304.3±17.61	138.1± 14.74***	781.0± 45.80***
H+Sim	241.9±35.43	104.3± 16.64*	652.7± 21.00

AST: aspartate transaminase (U/L), ALT: alanine transaminase (U/L); ALP: alkaline phosphatase (U/L). The results were analyzed using two-way ANOVA-Newman-Keuls multiple comparison test and expressed as mean ± S.E.M. ***The value is significantly different from normal rats plus saline group (P< 0.001, n = 7).

Highlights:

- *Guaraná reduced total cholesterol and LDL-C in hyperlipidemic rats.*
- *Guarana prevents memory impairment induced by hyperlipidemia*
- *Anxiety-like behaviors is not altered either by hyperlipidemia or by guarana, caffeine or simvastatin.*
- *Guarana prevented the alterations induced by hyperlipidemia in AChE activity in the hippocampus.*
- *Guarana could be used as adjuvant therapy in hyperlipidemic patients.*