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Influence of Moringa (*Moringa oleifera*) enriched ice creams on rats' brain: Exploring the redox and cholinergic systems

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ABSTRACT

The broad application of *Moringa oleifera* leaves in the treatment of numerous diseases is prevalent globally where it extends to the management of diabetes, hypertension, inflammation, hypercholesterolemia and neurodegenerative diseases. This study provides findings on the role of *Moringa oleifera* leaves (MO) [MO leaves] formulated ice creams on brain cholinergic enzymes [acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)], antioxidant enzymes, glycemic index and blood lipid profile of rats. Thirty (30) adult male rats acclimatized for 2 weeks were divided into five groups: Group 1 rats received commercial ice cream, Group 2 rats were received plain ice-cream, Group 3, 4 and 5 received 0.5 g, 1.0 g and 2.0 g of MO-formulated ice creams. Rats were fed on normal pellets and exposed to ice creams produced from whipping cream, skimmed milk and *Moringa oleifera* leaves for 30 consecutive days. Following administration, results from this study revealed that rats that received Moringa formulated ice-creams had reduced brain butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) enzymes activities, glycemic index (GI), total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) levels and significantly increased high-density lipoprotein-cholesterol (HDL-C) level in the plasma while revealing elevated brain antioxidant status (Superoxide dismutase (SOD) and Catalase (CAT)) when compared against rats consuming commercial ice creams. Therefore, results from this study attests to the intake of ice creams made from blends of Moringa leaves in the reduction of rats' body weight, glycemic index and lipid profile (TC, TG, LDL-C), inhibition of brain cholinergic enzymes (AChE and BChE) while increasing brain antioxidant enzymes activities (SOD and CAT).

1. Introduction

The Moringaceae family includes about 13 species and genus varieties of shrubs and trees cutting across Arabia, Africa and Asia (Olson, 2002). The most commonly studied and used species variant is *Moringa oleifera* which is enriched with high phytochemicals inclusive of but not limited to in its leaves, pods and seeds (Popoola and Obembe, 2013; Sivasankari et al., 2014). The use of *Moringa oleifera* extends to the management of diabetes, cancer, oxidative stress, inflammation, hypercholesterolemia activity (Vergara-Jimenez et al., 2017; Farooq et al., 2012) amongst other diseases. Unlike any other medicinal plant with single antioxidant effects, *Moringa oleifera* has aggregates of antioxidants and have been inferred to be more beneficial with identified polyphenols (Mishra et al., 2011; Tejas et al., 2012). It is to this end that *Moringa oleifera* leaf, seed and flower have found numerous applications in food and have been reported to be used as food fortified foods, amala (stiff dough), ogi (maize gruel), bread, biscuits, yoghurt, cheese and in making soups according to Oyeyinka and Oyeyinka (2018)

A high-calorie diet promotes hyperglycemia, increases fasting triacylglycerol and reduces high-density lipoprotein (HDL) cholesterol levels (Aryangat and Gerich, 2010). The measure of the glycemic index (GI) of food assists in assessing such foods blood glucose response (Jenkins et al., 1981). *Moringa oleifera* acts as a hypolipidemic agent, a mechanism established based on its inhibitory action on enzymes such as HMG-CoA reductase (Hassarajani et al., 2007), its abundance of β-sitosterol, bioactive compound (Halaby et al., 2013) and saponins (Oyedepo et al., 2013) which together reduces cholesterol level and increases enterohepatic circulation of bile acids thereby increasing cholesterol fecal excretion, biliary excretion and reduction in plasma cholesterol respectively. According to the French National Centre for Scientific Research (CNRS, 2014), the effects of consuming foods rich in polyunsaturated lipids assists in protecting against neuronal

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differentiation which contributes to cerebral ischemia. Additionally, in the instance that there is an aberration in the regulation of lipids metabolism, there is the occurrence of numerous brain diseases (Hussain et al., 2013).

Of the various food types, the intake of snacks and dairy products such as ice creams remain a challenge especially with concerns as to its effects on health and quality of life. However, fortification of ice creams with nutrients and/or bioactive substances can be beneficial (Anil and Singh, 2007). The application of *Moringa oleifera* to diet is a common trend globally. Notably, Gopalakrishnan et al. (2016) gave an account of *Moringa oleifera* inclusion in the diet of HIV positive individuals as it boosts their immune system making *Moringa oleifera* to be a promising neuroprotectant. Reports by Baker et al. (1998), Kirisattayakul et al. (2013) gave inciteful scientific claims of its action in depleting brain reactive oxygen species, and in improving antioxidants enzyme activities, neuroprotective and memory-enhancing effects (Sutalangka et al., 2013).

Interestingly, findings on the modulatory effects of ice creams enriched with *Moringa oleifera* leaves, [MO] -formulated ice-creams on brain antioxidant enzyme, cognitive-enhancing enzymes, glycemic indices and body weight of rats is limited. Hence, the objective of this study is to identify the role of MO formulated ice-creams on brain cognitive function, hypolipidemic and its antioxidant potentials in rats.

2. Methods

2.1. Sample and reagents

Moringa oleifera leaves were gotten from the Botanical Garden, The Federal University of Technology Akure, Nigeria. Commercially consumed ice cream was purchased from a commercial creamery besides The Federal University of Technology Akure, Nigeria while whipping cream and skimmed milk were purchased from the supermarket. The reagents such as 5,5′-dithio-bis-(2- nitrobenzoic) acid [3.3 mM, 100 μL) and 0.1 M phosphate buffer (pH 8.0) were all sourced from Sigma Chemical Co. (St. Louis, MO, USA). Other reagents used were of analytical grade.

2.2. Preparation of ice cream

The leaves of *Moringa oleifera* was washed and dried for 24 h at 70 °C. The dried leaves were milled to produce a free-flowing powder. The whipping cream and skimmed milk were purchased from a supermarket in Akure, Nigeria. The ice-cream product purchased (commercial ice cream) was from the creamery with production information revealing it to contain milk, cream, sugars, modifying agents, and artificial flavorings. However, further details were not provided for commercial reasons. The ice creams produced were different proportions namely; Plain-50 g whipping cream + 35 g skimmed milk, MO (0.5) – 49.5 g whipping cream + 35 g skimmed milk + 0.5 g moringa leaf powder, MO (1.0) - 49 g whipping cream + 35 g skimmed milk + 1 g moringa leaf powder and MO (2.0) - 48 g whipping cream + 35 g skimmed milk + 2 g moringa leaf powder respectively. To make the MO-formulated ice creams with *Moringa oleifera* leaves, firstly, whipping cream was whipped in a bowl using a hand mixer for 20 min. Thereafter, the skimmed milk and measured proportions of leaves of *Moringa oleifera* were added to the mixture, stirred further until a consistent texture is derived. Thereafter, the ice cream was scooped in plastic bowls and stored at −18 °C, in the freezer left to harden after 3 hrs.

2.3. Sensory evaluation

Sensory analysis was conducted to measure the acceptability of the ice creams formulations and was approved by the Center for Research and Development (CERAD), Federal University of Technology Akure. The test was done amongst 80 panelists across a wide range of age and gender. Importantly, the chosen panelists were selected based on their familiarity and are of the habit of consuming ice cream. They then scored each sample based on attributes which included taste, texture, color, aroma and overall acceptability between the scale range of 1–7 (where 1 is equivalent to very poor and 7 equates as excellent). Each time panelist consumed the formulated ice creams, water was provided for mouth rinsing. Also, spoons (plastics) used was changed before consuming the next formulated ice cream. The report of the sensory scoring is illustrated in Table 1.

2.4. Experimental design

Thirty mature male wistar Albino rats gotten from Animal house, Department of Biochemistry, Federal University of Technology Akure, Nigeria was used. These rats were divided into five (5) groups with weights between 240 and 250 g. The study commenced on approval by ethical committee of the Federal University of Technology, Akure, Nigeria. They were housed in plastic cages. The rats were maintained at 25 °C on a 12 h light/dark cycle with free access to standard pellet diet. The pellet diet contained 22.5 % protein, 64 % carbohydrate, 5 % fat, 3% fiber, and 5 % vitamins and minerals and water. The humidity was controlled. They were acclimatized under these conditions for 2 weeks prior to the commencement of the experiment. The experiment started after 14 consecutive days of acclimatization. Individual rat, housed separately was given 3 g of molted ice cream daily after produced ice creams was made to thaw. This was done by weighing ice cream in bowls, observing each rat's complete intake of the served ice-cream before serving rats standard pellet diet daily following the enlisted grouping:

- Group 1 - Rats were given commercial ice cream.
- Group 2 - Rats were given plain ice cream
- Group 3 - Rats were given MO (0.5) enriched ice cream.
- Group 4 - Rats were given MO (1.0) enriched ice cream.
- Group 5 - Rats were given MO (2.0) enriched ice cream (Fig. 1)

The exposure of rats to formulated ice cream diets at 12:00 noon each day lasted for 30 days. The weight of each rat was measured in grams daily on a weighing scale and reading taken when rat is stable. Fig. 2 shows the percentage weight gain of rats recorded for each experimental group. After which, experimental rats were sacrificed by cervical dislocation. The blood sample was collected and mixed with EDTA (concentration). The plasma was subsequently prepared and rats' brains dissected out and isolated by blotting on filter papers to remove adhering blood, rinsed in cold saline (0.9 %) and homogenized in

Table 1
Sensory analysis of formulated ice cream and commercial ice cream samples.

Sample	Texture	Taste	Colour	Aroma	Overall Acceptability
Commercial	6.10 ± 0.81 ^{d}	6.20 ± 0.74 ^{c}	6.38 ± 0.60 ^{d}	6.43 ± 0.99 ^{c}	6.57 ± 0.60 ^{d}
Plain	6.50 ± 0.66 ^{d}	6.50 ± 0.60 ^{c}	6.42 ± 0.99 ^{d}	6.39 ± 1.14 ^{c}	6.55 ± 0.82 ^{d}
MO (0.5 g)	5.48 ± 1.13 ^{c}	5.43 ± 1.21 ^{b}	5.74 ± 1.05 ^{c}	5.57 ± 0.94 ^{b}	5.83 ± 0.92 ^{c}
MO (1.0 g)	4.65 ± 1.27 ^{b}	4.83 ± 0.89 ^{b}	5.00 ± 1.02 ^{b}	4.96 ± 1.04 ^{a}	5.13 ± 0.92 ^{b}
MO (2.0 g)	4.39 ± 1.26^{a}	3.91 ± 1.12 ^{a}	4.83 ± 0.97 ^{a}	4.57 ± 1.32 ^{a}	4.61 ± 1.15 ^{a}

Values represent mean ± standard deviation.
Values with the same superscript number on the same column are not significantly ($P < 0.05$) different. Commercial- Commercial Ice cream; Plain-50 g whipping cream +35 g skimmed milk; MO [0.5]-49.5 g whipping cream + 35 g skimmed milk + 0.5 g moringa leaves powder; MO [1.0]- 49 g whipping cream + 35 g skimmed milk + 1 g moringa leaves powder; MO [2.0]- 48 g whipping cream + 35 g skimmed milk + 2 g moringa leaves powder.

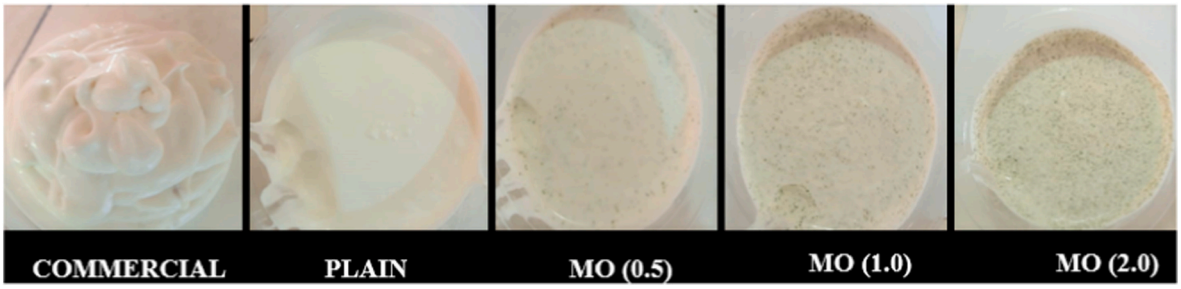

Fig. 1. Picture showing different MO formulated ice-creams produced. Commercial- Commercial ice cream; Plain-50 g whipping cream +35 g skimmed milk; MO [0.5]-49.5 g whipping cream + 35 g skimmed milk + 0.5 g moringa leaves powder; MO [1.0]- 49 g whipping cream + 35 g skimmed milk + 1 g moringa leaves powder; MO [2.0]- 48 g whipping cream + 35 g skimmed milk + 2 g moringa leaves powder.

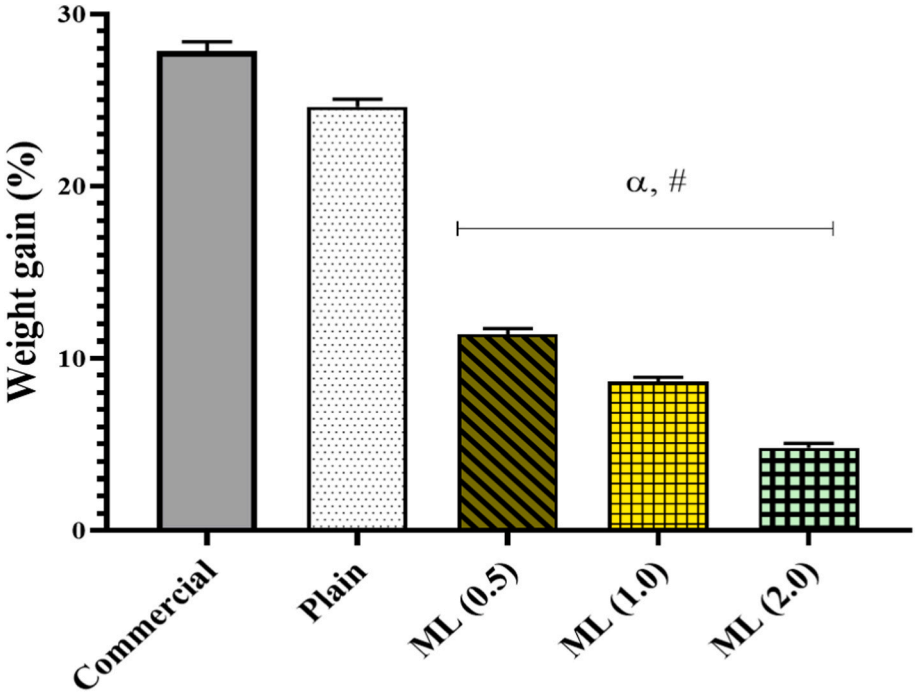

Fig. 2. Shows the effects of inclusion of different formulated ice-creams on the body weight gain (percentage) of rats. Data are presented as mean value ± standard error of mean (SEM), n = 6. Commercial- Commercial ice cream; Plain-50 g whipping cream +35 g skimmed milk; MO [0.5]-49.5 g whipping cream + 35 g skimmed milk + 0.5 g moringa leaves powder; MO [1.0]- 49 g whipping cream + 35 g skimmed milk + 1 g moringa leaves powder; MO [2.0]- 48 g whipping cream + 35 g skimmed milk + 2 g moringa leaves powder.

sodium phosphate buffer (0.1 M, pH 6.9). The homogenate was centrifuged at 10,000 × *g* for 10 min at 4 ° C, and the supernatant was used for subsequent biochemical assays.

2.5. Oxidative stress markers

The activities of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT)) and malondialdehyde (MDA) level in brain tissue homogenate were determined following the method of Akinyemi et al. (2017). The levels of rat plasma total protein (TP) estimated were determined using commercial colorimetric enzymatic diagnostic kits (Randox Laboratories Ltd., Crumlin, UK).

2.6. Lipid profile

The estimation of rats' lipids contents namely plasma total cholesterol (TC), triglyceride (TAG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) was in accordance to the of Friedewald et al. (1972) and carried out experimentally using Randox assay kits (Randox Laboratories Limited, Crumlin, United Kingdom).

2.7. Cholinergic enzyme activities

The enzymes activities [acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)] in the brain of experimental rats were measured using acetylthiocholine iodide and butyrylcholine iodide as substrates following the modified method of Li et al. (2000). The specific activity of AChE and BChE was expressed as μmol. AChE/h/mg protein and μmol. BChE/h/mg protein respectively at an absorbance of 412 nm.

2.8. Glycemic index (GI)

The glycemic index of ice cream samples was assessed according to the method of Adedayo et al. (2018) at an absorbance of 540 nm. 25 mg of samples was added to 5-ml stomach solution (KCl–HCl buffer, pH 1.5), 2.5-ml α-amylase solution, 500 μl sodium acetate pH 4.75, 5 μl of α-glucosidase solution, DNSA solution (200 μl). The estimated values were deduced by calculating the sum of area under curve for each sample was divided by the sum of area under curve for standard glucose and multiplied by 100.

2.9. Statistical analysis

All data were analyzed using GraphPad statistical package (Version

5). Significance level was accepted at $P < 0.05$ after Tukey's post hoc test using one-way ANOVA. The result was expressed as mean ± standard errors mean (SEM) from derived triplicate values.

3. Results

3.1. Sensory evaluation findings

The evaluation of produced ice-creams gave results on the taste, texture, colour, aroma and overall acceptability on all ice-cream portions (Table 1). Result from this study showed that the acceptability of plain and commercial ice creams to be the highest when compared across the groups. The least accepted was MO (2.0) at 4.61 ± 1.15. The best accepted MO formulated ice cream was MO (0.5) with recorded score of 5.83 ± 0.92 which is significantly higher when compared to the scores of other MO formulated ice-creams.

3.2. Findings on body weight gain and glycemic index

Fig. 2 represented the body weight gained in rats fed on different portion of ice-creams. The body weight gained of rats fed with commercial and plain ice creams was elevated. Notably, rat fed on MO (0.5), MO (1.0) and MO (2.0) formulated ice-cream showed significant reduction ($P < 0.05$) in body weight gained (percentage) respectively when compared against rats fed with commercial and plain ice creams. The effect of the intake of formulated ice creams from MO leaves on glycemic index of rats is represented in Fig. 3. The result of inclusion of ice creams to daily diet of rat led to a significant change across the groups. MO (0.5), MO (1.0) and MO (2.0) showed significant reduction ($P < 0.05$) in glycemic indices when compared to rats fed with plain and commercial ice-creams to be 41.5 %, 36 % and 31.5 % respectively.

3.3. Lipid profile findings

Fig. 4 A, B, 4 C and 4 D gives a report of the total cholesterol, triglyceride, HDL-C and LDL-C in rats fed with formulated ice creams for 30 days. Overall, ice-creams fortified with MO (1.0) and MO (2.0) was effective in elevating HDL-C and reducing the level of total cholesterol, triglyceride and LDL-C significant at ($P < 0.05$) as against plasma content of rats fed with plain and commercial ice-creams.

3.4. Biochemical findings

Furthermore, in Fig. 5A and B the result of the effect of MO formulated ice-cream diet on the activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in normal rats is represented. The activities of AChE and BChE was elevated in rats fed with plain and commercial diets. Formulated ice-creams at MO (2.0) significantly reversed ($P < 0.05$) BChE activity in the brain of normal rats while MO (1.0) significantly reduced AChE activity the most when results were compared amongst rats fed with MO formulated ice creams, commercial and plain ice creams.

The antioxidant markers, superoxide dismutase (SOD) and catalase (CAT) activities of rats' brain of normal rats fed with enriched ice creams is reported in Fig. 6 A and B. There was a significant increase in the activities of brain SOD and CAT in rats fed with MO (1.0) and MO (2.0) ice creams when compared against rats fed with plain and commercial ice creams. In addition, the brain malondialdehyde (MDA) content in rats placed on plain and commercial diet for 30 days was significantly elevated (Fig. 6 C). Interestingly, MO-formulated ice creams fed to normal rats at (0.5), (1.0) and (2.0) and plain ice creams showed non-significantly reduction in brain MDA when compared amongst the groups. Whereas, a significant reduction was observed amongst all other groups when compared with commercial ice cream treated rats.

4. Discussion

Globally, food sources with enriched protein and phytochemical content are readily used and accepted in the food and dairy industries since they cause little or no change to the colour of fortified products (Oyeyinka and Oyeyinka, 2018) rather, they have high commercial value. The leaves, peels, seeds, and flowers of the Moringa tree have found beneficial use in medicine as a neuroenhancer, food and cosmetics industry (Iqbal et al., 2021). In this study, the overall acceptability of MO formulated ice cream was satisfactory with an exception to MO (2.0) formulated ice cream produced which recorded the least acceptability sensory wise owing to its undesirable colour change and plausible high chlorophyll level. Interestingly, a high proportion of *Moringa oleifera*

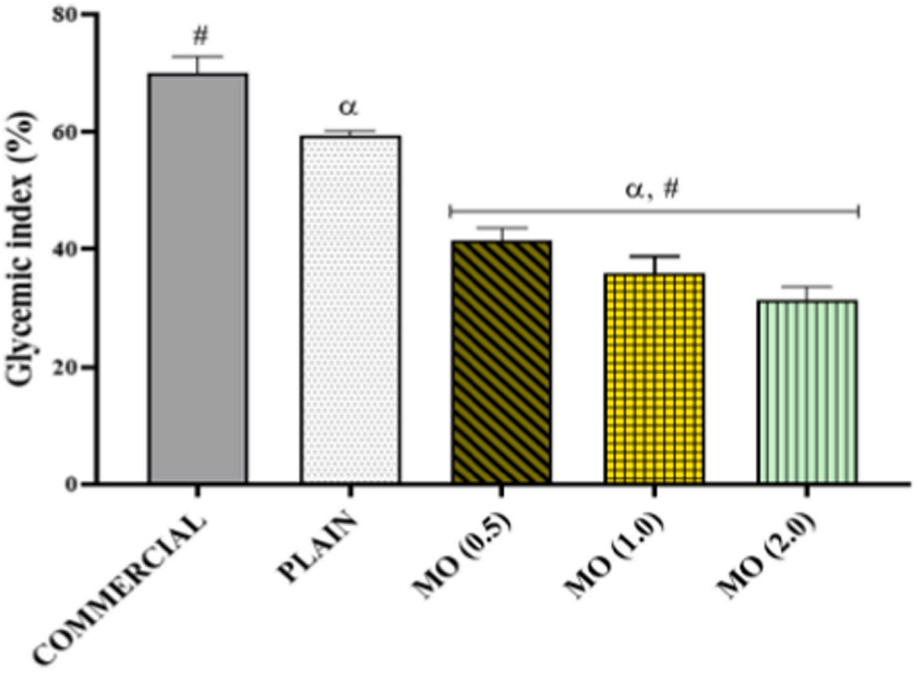

Fig. 3. Effect of the ice cream samples on rat glycemic Indices following consumption at different doses of ice -creams. Data are presented as mean value ± standard error of mean (SEM), n = 6. Values are statistically different with α as $P < 0.05$ versus commercial and values are statistically different with # as $P < 0.05$ versus plain ice cream respectively. Commercial- Commercial Ice cream; Plain-50 g whipping cream +35 g skimmed milk; MO [0.5]-49.5 g whipping cream + 35 g skimmed milk + 0.5 g moringa leaves powder; MO [1.0]- 49 g whipping cream + 35 g skimmed milk + 1 g moringa leaves powder; MO [2.0]- 48 g whipping cream + 35 g skimmed milk + 2 g moringa leaves powder.

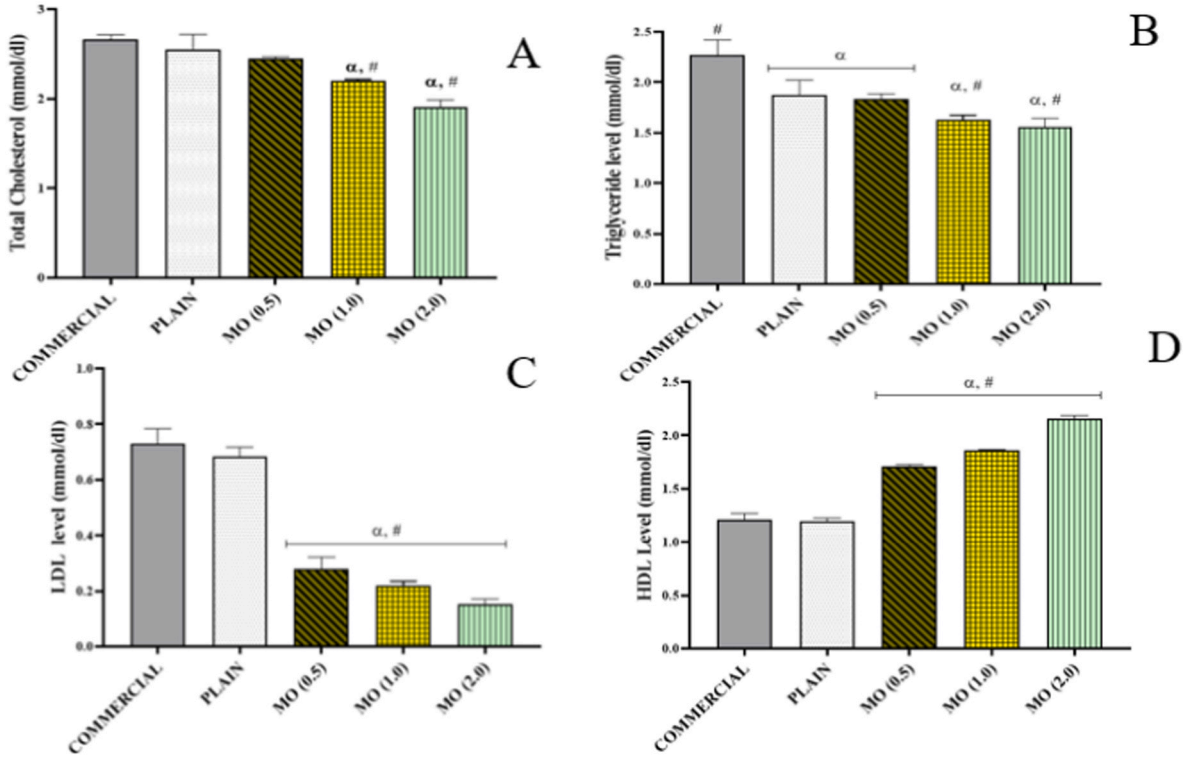

Fig. 4. Effect of the ice cream samples on plasma A) TC, B) TGA, C) HDL and D) LDL level following consumption at different doses of ice -creams. Data are presented as mean value ± standard error of mean (SEM), n = 6. Values are statistically different with α as $P < 0.05$ versus commercial and values are statistically different with # as $P < 0.05$ versus plain ice cream respectively. Commercial- Commercial Ice cream; Plain-50 g whipping cream +35 g skimmed milk; MO [0.5]- 49.5 g whipping cream + 35 g skimmed milk + 0.5 g moringa leaves powder; MO [1.0]- 49 g whipping cream + 35 g skimmed milk + 1 g moringa leaves powder; MO [2.0]- 48 g whipping cream + 35 g skimmed milk + 2 g moringa leaves powder; TC- Total cholesterol; TGA- Triglyceride; HDL-high density lipoprotein; LDL-low density lipoprotein.

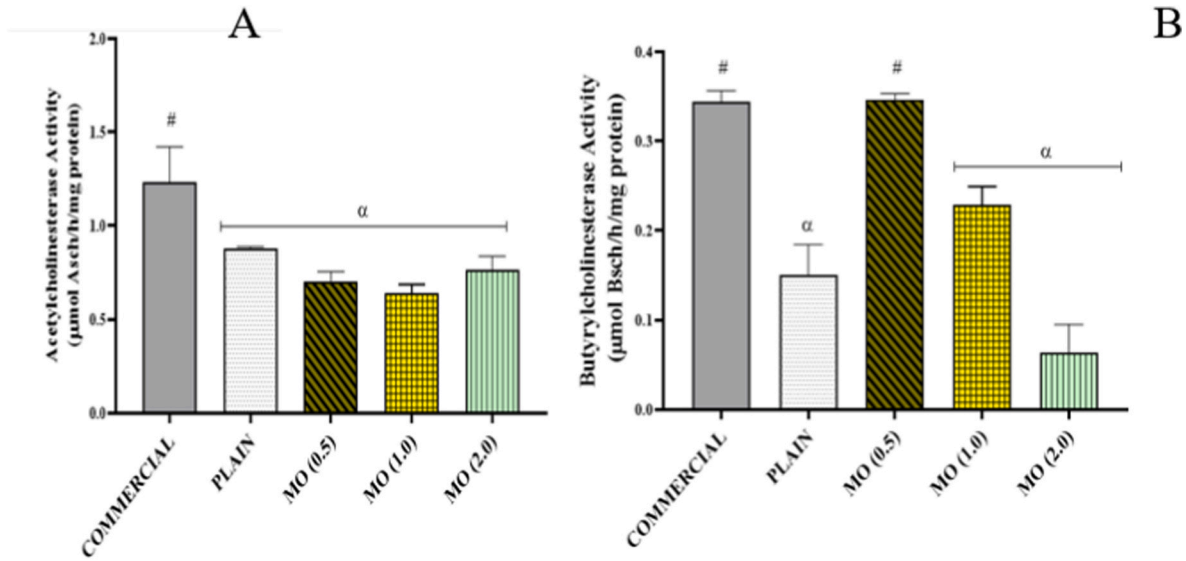

Fig. 5. Effect of the ice cream samples on rats' brain A) AChE and B) BChE activities following consumption at different doses of ice-creams. Data are presented as mean value ± standard error of mean (SEM), n = 6. Values are statistically different with α as $P < 0.05$ versus commercial and values are statistically different with # as $P < 0.05$ versus plain ice cream respectively. Commercial- Commercial Ice cream; Plain-50 g whipping cream +35 g skimmed milk; MO [0.5]-49.5 g whipping cream + 35 g skimmed milk + 0.5 g moringa leaves powder; MO [1.0]- 49 g whipping cream + 35 g skimmed milk + 1 g moringa leaves powder; MO [2.0]- 48 g whipping cream + 35 g skimmed milk + 2 g moringa leaves powder; AChE - Acetylcholinesterase; BChE - Butyrylcholinesterase.

leaves measured in food products gives an unappealing appearance as reported by Govender and Siwela (2020) owing to its high chlorophyll content (Karim et al., 2015). This can be inferred to also affect the sensory taste of produced MO (2.0) formulated ice cream in this study. Hence, increased concentration of *Moringa oleifera* leaves in MO (2.0) formulated ice-creams contributes to low sweetling and overall acceptability of its savory property.

Obesity emerges from excessive intake of fat, energy or energy

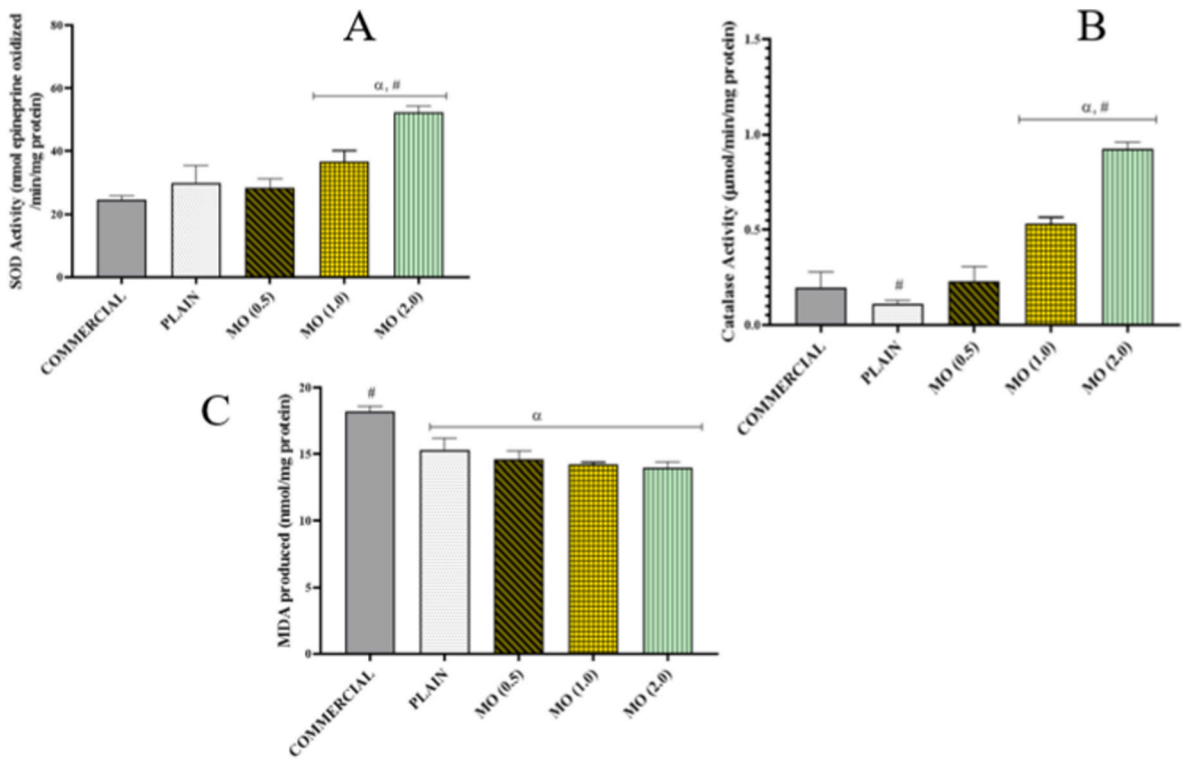

Fig. 6. Effect of the ice cream samples on brain A) SOD, B) CAT activities and C) MDA level following consumption at different doses of ice -creams. Data are presented as mean value ± standard error of mean (SEM), n = 6. Values are statistically different with α as $P < 0.05$ versus commercial and values are statistically different with # as $P < 0.05$ versus plain ice cream respectively. Commercial- Commercial Ice cream; Plain-50 g whipping cream +35 g skimmed milk; MO [0.5]- 49.5 g whipping cream + 35 g skimmed milk + 0.5 g moringa leaves powder; MO [1.0]- 49 g whipping cream + 35 g skimmed milk + 1 g moringa leaves powder; MO [2.0]- 48 g whipping cream + 35 g skimmed milk + 2 g moringa leaves powder; SOD- Superoxide dismutase; CAT- Catalase; MDA- Malondialdehyde.

enriching diet (Drummen et al., 2018). The role of excess calorie diet on the brain structural architecture is seen as a reduction in grey matter volume in several brain regions such as the hippocampus, prefrontal cortex, and other subcortical regions (Raji et al., 2010). Additionally, excess calorie intake causes detrimental effects leading to increased body mass index (BMI) thereby resulting in brain structural atrophy. Manning et al. (1997) and Manning et al. (1993) reported the contributing effect of glucose on the progression of cognitive impairment and/or memory deterioration in both healthy elderly individuals and patients with Alzheimer disease wherein, available free glucose causes detrimental effects to cognitive function. We reported a marked reduction in the body weight gained in rats consuming MO (2.0) formulated ice cream. The reduction of this ice cream on rats' body weight could be a result of the hypoglycemic potential of *Moringa Oleifera* (Kar et al., 2003). Foods with a high glycemic load (GL) contributes to an increased rate of released free glucose in circulation and the brain leading to a decline in cognition (Gentreau et al., 2020). It is thus reasonable to say that foods with low-GI induce ameliorative effects on damages related to brain cognitive function since their ability to generate free glucose is low. Notably, MO (2.0) formulated ice cream had the lowest glycemic index value hence, glucose is released at a markedly reduced rate by this ice cream in rats.

The hypocholesterolemia and hypolipidemic property of *Moringa oleifera* leaves have been reported by Chatterjee et al. (2013) and Shahinaz et al. (2017). *Moringa oleifera* has high flavonoids (Youdim and Joseph, 2001) and polyunsaturated fatty acids in its leaves as high as 76% in proportion (Yang et al., 2006). These polyunsaturated fatty acids, PUFAs assist in mopping cholesterol known to cause detrimental effects when circulated to the brain (Shahinaz et al., 2017). Sutalangka et al. (2013) attest to the flavonoids present in *Moringa oleifera* leaves extract to be neuroprotective and in enhancing cognitive function in rats. Rats fed with MO fortified ice-creams showed significantly reduced circulating total cholesterol (TC), triglycerides (TG) and LDL-cholesterol (LDL-C) in the blood which is inclusive of the brain as described by Assini et al. (2013) by mopping bad cholesterol in the brain. Therefore, MO formulated ice-creams can be said to have neuroprotective properties (Sun et al., 2008) as a result of its high phenolic content and anti-hyperlipidemic property as it exhibits a property similar to PUFAs in depleting deleterious high cholesterol.

The availability of phytonutrients; carotenoids, tocopherols and ascorbic acid (Saini et al., 2014), β-carotene (Lopez-Teros et al., 2017), tannins, saponins, flavonoids, terpenoids and glycosides polyphenolic compounds (Mishra et al., 2011; Lopez-Teros et al., 2017) in *Moringa oleifera* leaves have been thoroughly reported. Taken together, this facilitates its tendency to remove free radicals thereby neutralizing singlet or triplet oxygen, or peroxides in the system (Pokorny et al., 2001; Zheng and Wang, 2001; Sun et al., 2008) and confirms the fact that *Moringa oleifera* leaves is a potent antioxidant (Saini et al., 2014). Brain oxidative burden leads to free radicals-induced mitochondrial damage and neuronal cell death (Bhat et al., 2015; Mishra et al., 2011) seen in age-related cognitive depletion and neurodegenerative diseases progression. In the instance where the amount of MDA produced in biological tissue is overproduced, it is indicative of oxidation of circulatory lipids in the brain termed lipid peroxidation. Rats consuming MO formulated ice cream showed significantly elevated SOD and CAT antioxidant ability and reduction in the amount of malondialdehyde (MDA) produced in the brain of rats, therefore, disclosing its functional role as a neuroprotective agent as reported by Sutalangka et al. (2013) on account of its antioxidative potential (Saini et al., 2014).

Both brain oxidative burden (Mattson, 2004) and disturbances in neurotransmission, particularly cholinergic transmission contribute to cognitive dysfunction (Sutalangka et al., 2013). To illustrate, neurodegenerative diseases namely Alzheimer disease, Parkinson and dementia are characterized by impaired learning, memory function (Rubio et al.,

2017) and increased activities of cholinergic enzymes (ChEs) acetylcholinesterase and butyrylcholinesterase and excessive free radicals' generation (Sun et al., 2008). Medicinal plants with plausible effects in preventing abnormal hydrolysis of acetylcholine and butyrylcholine neurotransmitters are beneficial in managing impaired cholinesterase (ChEs) neurotransmission (Nwanna et al., 2016). In this study, there were elevated activities of AChE and BChE enzymes in the brain of rats fed with commercial and plain ice-creams. Conversely, the reversal effect of MO formulated ice-creams was shown with depleted AChE and BChE activities in the brain of rats owing to its high phytochemical constituents in the leaves of *Moringa oleifera* as reported by Ndong et al. (2007) in addition to its antioxidant properties (Saini et al., 2014).

5. Conclusion

Observations from this study showed that formulated ice creams from blended leaves of *Moringa oleifera* (MO) reversed elevated brain cholinergic enzymes, estimated body weight gained, glycemic index, the concentration of total cholesterol, triglyceride, LDL-C and brain malondialdehyde produced. However, the level of HDL-C, brain antioxidant activities was elevated in normal rats fed with MO-enriched ice-creams. The role of MO leaves enriched in ice-creams on lipid profile indices and brain enzyme markers explore its medicinal application in the ice-cream diet as a promising functional food in managing brain antioxidant status and cognitive enzymes activities.

Availability of data and material (data transparency)

The data that support the findings of this study are made available by contacting the corresponding author (ayoademosun@yahoo.com).

Code availability (software application or custom code)

Not Applicable.

CRediT authorship contribution statement

Ayokunle Olubode Ademosun: Conceptualization, Study Conception and design, Acquisition of data, Drafting of Manuscript. **Ganiyu Oboh:** Conceptualization, Study Conception and design, Acquisition of data, Drafting of Manuscript, Critical revision. **Olufunke Florence Ajeigbe:** Conceptualization, Study Conception and design, Drafting of Manuscript, Critical revision.

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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