Lycopene Supresses Palmitic acid-induced Neuro-oxidoinflammation via Attenuation of Oxidative stress and NF-κB-p65 activation in Female Rats

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Abstract

Neuroinflammation can be triggered by certain nutrients. Effect of lycopene against palmitic acid-induced neuroinflammation in female rats has not been explored. This study evaluated the effects of lycopene against palmitic acid (PA)-induced neuroinflammation in rats. Thirty rats (weighing 150–200 g) were randomised into six groups (n = 5): Normal control, PA control, PA + lycopene (0.24 mg/kg), PA + lycopene (0.48 mg/kg), lycopene (0.24 mg/kg), and lycopene (0.48 mg/kg), respectively. After seven weeks of PA challenge including two weeks of lycopene treatment, brain was excised for analyses. The PA-induced significantly ($p < 0.05$) increased adenosine deaminase, monoamine oxidase-A, nucleotides tri-phosphatase, 5'-nucleotidasea, acetylcholine esterase, myeloperoxidase activities, and malondialdehyde level, reduced significantly post-treatment. Conversely, catalase and glutathione peroxidase activities, and reduced glutathione levels decreased (PA control) by 43%, 34%, and 12%, respectively, compared with the Control. Also, PA triggered a decrease in the brain phospholipids (11.43%) and cholesterol (11.11%) levels, but increased triacylglycerol level (50%). Furthermore, upregulated expressions of IL-1β, IL-6, and NF-κB-p65 in the PA control were attenuated, while decreased IL-10 was upregulated after treatment. Severe vacuolation in PA control was normalized by lycopene. This study concludes that, lycopeneameliorated PA-induced neuroinflammation, probably via attenuation of oxidative stress, and downregulation of TLR4/ NF-κB -p65 axis.

Introduction

Neuro-inflammation (arising from meta-inflammation) describes an immuno-metabolic disorder of the nervous system, arising from derangements of the metabolic and inflammatory pathways in response to various cues including infection, autoimmunity, nutrients, or toxic metabolites (Tomassoni *et al*., 2020). Nutrients (such as free fatty acids) may accumulate in the nervous tissue such as the microglia due to excessive positive energy balance. The sustained impaired energy balance may invoke glial cells activation and disproportionate production of reactive oxygen species (ROS). Either of the generated ROS or free fatty acids, such as palmitic acid can activate the canonical inflammatory pathways, and cause chronic inflammation of the brain (Kozicz and Morava, 2018). The chronic inflammation may later degenerate into a myriad of neuro-psychopathologies, including major depressive disorder, Parkinson's, and Alzheimer's diseases (Tommassoni *et al*., 2020). The neuropathology is usually characterized by disturbances of neuro-behavioural enzymes such as acetylcholine esterase, monoamine oxidase- A, and adenosine deaminase (Avci and Durak, 2008; Cavalcante *et al*., 2020).

Excessive intake of palmitic acid (PA)- a long-chain saturated fatty acid, and an agonist of the immune-modulating toll-like receptor-4 (TLR-4), may orchestrate the downstream cross-talks among many adaptor proteins, consequently activating the nuclear factor kappa B (NF-κB) via the MyDD8/TRAF/IKK pathways (Gaber *et al*., 2017). NF-κB activation often induces hyper-proliferation of pro-inflammatory cytokines such as interleukin-1beta (IL-1β), and interleukin-6 (IL-6), and usually parallels with the downregulation of anti-inflammatory phenotypes such as interleukin-10 (IL-10) (Dali-Yousef and Ricci, 2015). The hyper-proliferation and infiltration of these pro-inflammatory mediators and high metabolic demand of the brain

predisposes it to oxidative stress, which exacerbates the neuroinflammation (Camandola and Mattson, 2017). Oxidative stress-induced neuroinflammation alters neurotrophic factors levels, disrupts neurotransmission balance, and contributes to neuronal damage and cognitive deficit (Tangvarasittichai, 2015; Chen *et al*., 2019; Tomassoni *et al*., 2020). Moreover, dysregulation of lipids, such as triacylglycerol and cholesterol correlates positively with neurodegenerative diseases (Chen *et al*., 2019). Understanding the aetiology of neuro-inflammation from the energy homeostasis standpoint is of great importance to long-term prevention of neuro-pathologies. More so, pharmacological intervention remains a therapeutic possibility in neuropathic management (Kozicz and Morava, 2018).

Lycopene ($C_{40}H_{56}$), an aliphatic polyunsaturated carotene-containing compound, has been classified as a functional food and is abundant in tomatoes, watermelons, papaya, and guava (Chen *et al*., 2019). Believed to be one of the most potent anti-inflammatory phytochemical, it also abrogates oxidative stress via chain-breaking mechanisms and electron donation *in vivo*. It has been shown that lycopene has modulatory activities against various neurodegenerative diseases (Yu *et al*., 2017). In spite of this, there is dearth of information on possible neuroinflammatory-inducing effect of palmitic acid in female rats. Besides, experimental evidence also, suggests that obesity is more prevalent in females, who are also more susceptible to inflammation-related diseases relative to their male counterparts worldwide (Card et al., 2006; Umuerri et al., 2017). Furthermore, a review of literatures shows that females are more prone to neuroinflammatory disorders, such as Alzheimer's disease, amyotrophic lateral sclerosis, and depression (Hanamsagar and Bilbo, 2016). This study, therefore, aimed to investigate the neuroinflammatory-inducing effect of PA in female Wistar rats, and explore the possible therapeutic effects of lycopene against this disorder.

2.0 Materials And Methods

2.1 Chemicals and reagents

Lycopene (All-*trans*) and TRIzol were purchased from Solarbio Life Science and Co. Ltd. Beijing, China. Palmitic acid, fatty acid–free bovine serum albumin (BSA), Guaiacol, adenosine triphosphate (ATP), acetylthiocholine iodide, adenosine, 5'-5'-dithiobis- (2-dinitrobenzoic acid) (DTNB), and Pyrogallol are products of Sigma Aldrich (St. Louis, Missouri, United States). All other chemicals used were of pure and analytical grade. The relative expressions of mRNA coding for IL-1β, IL-6, IL-10, NF-κB -p65, and GAPDH were quantified using the primer sequences synthesized by ShineGene Bio-Technologies, Inc. Shanghai, Xuhui District (China). Total cholesterol, triacylglycerol, and phospholipids kits were products of Labkits® Diagnostics Company (Barcelona, Spain).

2.2 Methods

2.2.1 Experimental animals

Thirty (30) female Wistar rats weighing between 150 - 200 g were purchased and kept in the Animal Housing Unit of the Department of Biochemistry, Federal University of Agriculture, Abeokuta, Ogun State,

Nigeria. The animals were acclimatized for two weeks before the commencement of the experiment. They were housed in plastic cages with good ventilation and supplied with standard pellets and clean water ad *libitum*. All animals were handled humanely according to the guidelines for use of experimental animals (Sherwin *et al*., 2003). Ethical approval (FUNABCH170641) was obtained for the study from the Departmental Ethical Committee.

2.2.2 Induction of neuroinflammation and experimental design

Neuroinflammation was induced via administration of 5mM PA (i.p.) for five weeks. PA was complexed with 1% BSA and given five times weekly at 100 µL/100 g body weight. The ratio of palmitic acid (PA) to BSA was 8:1 (Tashiro *et al*., 2017). Briefly, 50 mM PA (stock solution) was initially dissolved in 50% ethanol and incubated at 60° C and later brought to 5 mM using the 1% BSA (dissolved at 35 ºC) (Wang *et al*., 2017). The resulting PA-BSA complex was kept at -20 ºC and thawed when needed. After the first five weeks, animals were grouped and treated as shown in Table 1 (without halting PA administration together with the lycopene treatment), for two weeks. The study was for seven weeks. The lycopene was reconstituted in olive oil and administered to the animal by oral gavage. The dose of lycopene chosen was based on the previous study (Devaraj *et al*., 2008). Although, there is currently no standardized therapeutic dose for lycopene, a dose of 7-21 mg/70kg/ day was believed to be beneficial (Devaraj *et al*., 2008), and is equivalent to 0.24 mg/ kg body weight of the rats, consequently, graded doses of the 0.24 and 0.48 mg/kg was used for the study.

Table 1: Experimental design

Groups	First 5 weeks*	Treatment(last 2 weeks)
Normal control	1% BSA	+ vehicle
Palmitic acid (PA)	5 mM PA + 1% BSA	+ vehicle
PA + Lycopene (0.24 mg/kg)	5 mM PA + 1% BSA	+ lycopene (0.24 mg/kg)
PA + Lycopene (0.48 mg/kg)	5 mM PA + 1% BSA	+ lycopene (0.48 mg/kg)
Lycopene (0.28 mg/ kg)	1% BSA	+ lycopene (0.24 mg/kg)
Lycopene (0.48 mg/kg)	1% BSA	+ lycopene (0.48 mg/kg)

***The respective PA and/or BSA administration lasted throughout the study; BSA = Bovine serum albumin; PA = palmitic acid; vehicle = olive oil

2.2.3 Animal sacrifice and tissue preparation

After the end of seven weeks, the animals were fasted overnight and sacrificed by cervical dislocation. Brain tissue was excised, blotted dry, and preserved at -20°C prior to biochemical analyses.

2.2.4 Preservation of tissues for histology and gene expression analyses

A section of brain tissue was fixed in 10% formalin for histological assessment, while a small portion of the brain was preserved in 100 µL of TRIZOL reagent for gene expression analyses. The gene expression samples were kept -20°C until used.

2.3 Biochemical analyses

2.3.1 Assay for neuro-behavioural marker enzymes

The Acetylcholine esterase (AchE) activity was determined as briefly described. A working reagent (2 mL total volume) containing, 0.1M potassium phosphate buffer, pH 7.5, and 1 mmol DTNB. The method based on the formation of the yellow anion; 5, 5-dithiol-bis-acid-nitrobenzoic, measured by absorbance at 412 nm for 3 min. The reaction was initiated by addition of 0.8 mM acetylthiocholine iodide to the sample in the working reagent. The enzyme activity was expressed in nmol AChE/min/mg protein (Akinyemi *et al*., 2016).

Monoamine oxidase-A (MAO-A) activity was estimated using benzyl-amine (Akinyemi *et al*., 2016). Briefly, the reaction mixture 250 µL (containing 0.1M phosphate buffer, pH 7.4, 200 µM benzyl-amine, and 40 µL of the sample) was incubated for 1 hour at 37 ℃ and later cooled on ice. Then, 500 µL of distilled water, 250 µL of 10% zinc sulphate, and 50 µL of 1M NaOH was added, incubated for 2 minutes on the ice, and centrifuged (10, 000 g for 10 min). Finally, the supernatant was diluted five times with 1M NaOH, and the absorbance read at 450 nm.

Adenosine deaminase (ADA) activity was assayed for, as a direct measurement of the formation of ammonia produced when adenosine deaminase reacts with excess of adenosine (Akintunde *et al*., 2018). Concisely, 50 µL of the sample was reacted with 21mM of buffered adenosine; pH 6.5, incubated for 1 hour at 37°C. 300 µL of phosphate buffer was added to 200 µL of 0.15 mmol/L of ammonium sulphate solution. The mixture incubated for 30 minutes at 37º C followed by addition of 1 mL of phenol/ sodium nitroprusside (106/ 0.17 mmol/L) to the tubes to terminate the reaction. Then 1 mL of 125 mmol/L NaOH was added to the tubes followed by incubation at 37º C for 30 min, and, the absorbance monitored at 630 nm.

Nucleotide triphosphatase (NTPDase) activities were determined in the brain homogenate by NTPDase enzymatic assay was carried as described by Schetinger *et al*. (2007). A reaction medium (containing 5 mM KCl, 1.5 mM $CaCl_2$, 0.1 mM EDTA, 10 mM glucose, 225 mM sucrose and 45 mM Tris–HCl buffer, pH 8.0), in a final volume of 200 µL. 20 µL of sample was added to the reaction mixture and pre-incubated at 37ºC for 10 min. The reaction was initiated by the addition of 10 µL of ATP (1.0 mM) and incubation allowed to proceed for 20 min. The reaction was terminated by adding 100 µL of 5% SDS and the amount of inorganic phosphate liberated quantified as described by Katewa and Katyare (2003). Briefly, 750 µL of diluted 1.25% (w/v) ammonium molybdate [10 µl of 2.5 % ammonium molybdate (prepared with 3 N H_2SO_4) in 65 µl of 1.062 N H_2SO_4] was added to the mixture, followed by 100 µL of reducing agent (20 mg each of hydralazine sulphate and ascorbate in 1 ml of 0.1 N H_2SO_4). The whole reaction mix was

thoroughly mixed, and the absorbance measured at 820nm against blank within 30 min. The activity of NTPDase in the sample was extrapolated from the phosphate standard curve.

5’ nucleosidases (5-NTD) activity was estimated as per the method of El-Asser and El- Merzabani (1975). Briefly, 70µL of assay buffer (containing 62.5 mM Tris/HCl pH 7.4 and 62.5 mM $MgCl_2$) and 10 µL of freshly prepared adenosine monophosphate (AMP) and 100 mM L-cysteine (10 µL) was pipetted into well-labelled Eppendorf tubes and allowed to equilibrate at 37°C for 5 minutes. Afterwards, samples (10 µL) was added, vortexed thoroughly and incubated at 37°C for 45 minutes. The reaction was terminated by adding 5% SDS (50 µL) and the amount of inorganic phosphate liberated quantified as described by Katewa and Katyare (2003).

2.3.2 Assay for antioxidant and oxidative stress markers

The superoxide dismutase (SOD) activity was based on the inhibition of the auto-oxidation of pyrogallol according to Marklund and Marklund (1974). Briefly, 20 µL of the sample was allowed to incubate with 180µL of 100 mM Tris-HCl buffer, pH 8.2 for 10 minutes. Then, 50 µL of 10 mM pyrogallol was added to initiate the reaction, monitored for 3 minutes at 420 nm.

Catalase (CAT) activity was assayed for as described by Shangari and O’Brien (2006), based on the yellow complex formation between the excess hydrogen peroxide and ammonium molybdate at physiological pH. Briefly, 0.1 ml of the sample was incubated with 1 mL of buffered hydrogen peroxide (0.1 M phosphate buffer, pH 7.4 and 65 µL/mL), after 1 minute, the reaction was terminated with 32.4 mM of ammonium molybdate, and the absorbance read at 405 nm.

Glutathione peroxidase (GPX) activity was assayed for based on the conjugation of 5’-5’-dithiobis- (2-dinitrobenzoic acid) (DTNB) with the excess thiol group after the sample has been precipitated with 10 % TCA (Rotruck *et al*., 1973). In short, 25 µL of the homogenate was added to 75 µL GPx working reagent (containing 4mM GSH, 1mM NaN3, 2.5 mM H2o2, and 0.1 M phosphate buffer, pH 7.4) and allowed to stand for 10 minutes. Later, 25µL of 10% TCA was used to terminate the reaction and centrifuged at 3500 rpm for 10 minutes. 35 µL of the supernatant was reacted with 350 µL of GSH working reagent (containing 10mM DTNB and 0.3 M Tris- HCl buffer containing 1mM EDTA). The absorbance was taken 10 minutes later at 412 nm.

Myeloperoxidase (MPO) activity was assayed for in the post-nuclear fraction of the brain tissue as described by Klebanoff *et al*. (1984) with slight modifications. Briefly, 10µL of the homogenate was reacted with 10 µL of 4 M guaiacol and 100 mM H2O2 in 0.1 M phosphate buffer, pH 7.0 and the change in absorbance were monitored for a minute at 470 nm. The lipid peroxidation (MDA) level was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS), according to the method of Buege and Aust (1978). 0.5ml of sample (distilled water for blank) was treated with 1.0 ml of TBA reagent (containing 0.35 % TBA, 25mM HCl, and 15 % TCA) and was incubated in boiling water bath for 15min. The tube was immediately placed on ice to cool, centrifuged at 3500rpm for 10min and the absorbance of clear supernatant measured against blank at 532 nm.

Reduced glutathione (GSH) level was assayed according to Ellman (1959) method. Briefly, 25 µL of the sample was precipitated using 10% TCA. Then, the homogenate was centrifuged at 3500 rpm for 10 minutes. 35µL of the supernatant was then reacted with 350 µL of GSH working reagent (containing 10mM DTNB and 0.3 M Tris- HCl buffer containing 1mM EDTA), the absorbance read at 412 nm.

2.3.3 Brain lipids level determination

Brain triacylglycerol (TAG), cholesterol (CHOL), and phospholipids (PHOL) were evaluated after the lipid was extracted from the brain tissue as described previously (Ugbaja *et al*., 2016) using the commercial test kits. Briefly, 0.2 g of the brain was homogenized in chloroform/methanol mixture (2:1), and centrifuged at 3000 rpm for 10 minutes. The chloroform layer (500 µL) was then transferred into another Eppendorf tube, dried on water bath at 60 °C. Trixton –X (10µL) was added to the extract to precipitate any protein and dried again. The lipid content (TAG, CHOL, and PHOL) was then estimated with the test kits according to the manuals.

2.4 Gene expression analyses of the inflammatory markers

Brain total mRNA was extracted in the TRIzol, after which the relative expression of genes coding for interleukin 1-beta (IL-1β), interleukin 6 (IL-6), interleukin 10 (IL-10), and NF-κB, were normalized with GAPDH. The mRNA was converted to cDNA, and amplified using EasyScript one-time RT PCR Supermix (Cat No: AE411), produced by TransGene Biotech Co. Beijing, China. Primer sequences for the target genes are shown in Table 2

Table 2: Gene target primer sequences

Gene Target	Forward sequences	Reverse sequences
NF-κB-p65	TCCCACAAGGGGACATTAAGC	CAATGGCCTCTGTGTAGCCC
IL-1β	CCTTTGGCAAGTGTCTGAAGC	TCAGACAGCACGAGGCATTT
IL-6	TCCGGAGAGGAGACTTCACA	GAATTGCCATTGCACAACTCTT
IL-10	TGCGACGCTGTCATCGATTT	GTAGATGCCGGGTGGTTCAA
GAPDH	AGTGCCAGCCTCGTCTCATA	GATGGTGATGGGTTTCCCGT

2.5 Histological evaluation of the brain

A small portion of the brain tissue fixed in 10% formalin was cut (about 3 µm) and stained with Hematoxylin and Eosin, and later viewed under a microscope (Sahin *et al*., 2014).

2.6 Statistical analysis

Data are expressed as mean ± standard error mean (SEM). One-way Analysis of variance (ANOVA) was used to compare means across groups. Where heterogeneity occurred, Duncan Multiple Range Test (DMRT) was used to separate the means with ($p < 0.05$) considered significant. All these analyses were done using Statistical Package for Social Sciences (SPSS) version 20.0. The band densities of the gene expression analyses were quantified using Image-J (Schneider *et al.*, 2012), and were plotted as a bar graph using Graph Pad Prism (version 6.0).

3.0 Results

3.1 Lycopene abolishes derangements in brain neuro-behavioural enzymes activities

Palmitic acid (PA) caused a significant ($p < 0.05$) increase in the activity of AchE (19.28 %) in the brain when compared with the control group (Fig. 1A). Nevertheless, the activity was abated in groups treated with 0.48 mg/kg lycopene (23.60%) relative to the PA control group. ADA activity (Fig. 1B) increased dramatically (by 133.3 %) in the PA – treated group. The same trends were observed, for the activities of MAO-A (23.59%) and NTPDase (15.35%) Regardless, 0.48 mg/kg of lycopene significantly lowered the activities of MAO-A (26.78%) (Fig. 1C) and NTPDase (18.96%) (Fig. 1D) as well as ADA (28.57%). There was no significant difference between the groups treated with lycopene alone (0.24 mg/kg and 0.48 mg/kg) (Fig. 1) and the Normal control.

3.2 Lycopene abated palmitic acid-induced oxidative stress in rat brain

Following two weeks intervention with lycopene, the increased activities of 5-NTD (15.35 %) and MPO (232.84%), following PA challenge reduced significantly ($p < 0.05$) by 18.96% and 62% respectively by 0.48mg/kg lycopene. Similarly, MDA level increased (57.63%) significantly ($p < 0.05$) in the PA control group when compared with normal control. There was no significant difference in the 5-NTD and MPO activities between the two lycopene only doses (0.24- and 0.48 mg/kg) compared to the control group (Fig. 2).

3.3 Lycopene improves antioxidant markers in rats challenged with palmitic acid (PA)

There was no significant difference ($p > 0.05$) in the SOD activity (Fig. 3A) across the groups. The specific activities of GPx, CAT, and the level of GSH of the brain of the rats decreased significantly ($p < 0.05$), by 43%, 34 %, and 12 %, correspondingly in the palmitic acid- challenged group when compared with the control group (Fig. 3). Lycopene -supplemented groups showed a remarkable increase in the downregulated enzymes in a dose-dependent manner. Commendably, the group challenged with PA but treated [PA + lycopene (0.48 mg/kg)] showed more improved activities of GPx (Fig. 3B) and CAT (Fig. 3C)

relative to the Normal control group. However, there was no significant difference in GSH level (Fig. 3D) between the PA + lycopene (0.48 mg/kg) group and Normal control as is SOD activity (Fig. 3A).

3.4 Palmitic acid (PA) - mediated derangement of lipid metabolism in the rat brain was remediated by lycopene

Phospholipids (Fig. 4B) and cholesterol (Fig. 4C) levels were significantly ($p < 0.05$) reduced by 11.43% and 11.11% respectively in the PA-exposed rats, while triacylglycerol (Fig. 4A) by (50%) increased slightly when compared with the control group. Regardless of the perturbations observed in the PA control group, lycopene intervention reversed the disturbances in all the lycopene treated groups. There was no significant difference in the level of TAG (Fig. 4B) among the entire lycopene- treated group.

3.5 Lycopene modulated the induction of brain inflammatory cytokines in palmitic acid- exposed rats

Hyper-activation of brain NF-κB-p65 is a hallmark of palmitic acid group (Fig. 5D) when compared with the Normal control group. The relative expression of interleukin − 1beta (IL-1β) and interleukin- 6 (IL-6) similarly increased in the PA-untreated group, while interleukin-10 (IL-10) relative expression significantly decreased in the PA-untreated group. Lycopene supplementation significantly ($p < 0.05$) upregulated the expression of IL-10 (Fig. 5A), downregulated IL-1β (Fig. 5B), IL-6 (Fig. 5C), and NF-κB-p65 (Fig. 5D). The downregulation occurred in a dose-dependent manner. There was no significant difference between the two doses lycopene only groups (0.24- and 0.48 mg/kg) (Fig. 5).

3.6 Lycopene improves histo-morphological alterations of rats challenged with palmitic acid.

In Fig. 6, histological evaluation of the brains shows that palmitic acid (Fig. 6B) caused severe vacuolation when compared with the normal control (Fig. 6A) where there was moderate vacuolation. The histology of the groups treated with lycopene appeared to improve the brain architecture with the group co-treated with PA and lycopene (0.24 mg/kg) showing a normal histology (Fig. 6C) while the other lycopene- treated groups showed mild vacuolation (Figs. 6D, 6E, and 6F).

4.0 Discussion

In this study, PA administration caused the elevation of acetylcholine esterase, adenosine deaminase, monoamine oxidase- A, and nucleotide triphosphatase activities. Acetylcholine esterase is an enzyme involved in the breakdown of acetylcholine, a parasympathetic neurotransmitter into choline and acetate at the post-synaptic junctions of the central nervous system (Cavalcante *et al.*, 2020). Inhibition of acetylcholine esterase causes the accumulation of acetylcholine at the post-synaptic cleft, causing overstimulation of cholinergic responses. Overstimulation of the neuron may cause life-threatening irreversible brain damage (Cavalcante *et al.*, 2020). Contrariwise, hyperactivity of acetylcholinesterase is prodromal to Alzheimer's disease (AD); a devastating neurodegenerative disease characterized by impaired learning ability and memory loss (Nwanna et al., 2019). We observed that treatment with

lycopene in our study lowered the activity of acetylcholine esterase. The modulation of acetylcholine esterase activity by lycopene was previously reported following BPA-induced intoxication in rats (El-Morsy and Ahmed, 2020). Similarly, lycopene was shown to ameliorate Scopolamine induced amnesia by lowering the activity of AchE in mice (Bala et al., 2015). The neuroprotective effect of lycopene might be due to its ability to permeate the blood-brain- barrier and scavenging of ROS (Rao and Rao, 2007), or attenuation of oxidative stress (Bala et al., 2015). Furthermore, the ability of lycopene to augment brain-derived neurotrophic factors (BDNF) level via up-regulation of TrKB/MAPK/ERK1/2/CREB/BDNF signalling pathways is another possible underlying mechanism (Wang et al., 2016).

Adenosine deaminase (ADA) is a mammalian purine metabolizing enzyme, whose activity is critical in the clearance of adenosine- a regulatory metabolite with an anti-inflammatory function. ADA acts by converting adenosine to inosine, via the removal of the amino group of the adenosine (Dangana et al., 2019). The intracellular level of adenosine is strictly regulated by, and linked to energy status in the tissue (Antonioli *et al.*, 2012). In this context, the catabolic role of ADA is critical to its immunomodulatory effects, thus, making it a therapeutic target for inflammatory diseases (Cristalli *et al.*, 2001; Antonioli *et al.*, 2012). Although, the implication of ADA in neuro-degeneration is not clearly understood, activation of the T- cells by adenosine suggests that aberrant catabolism of adenosine by ADA may induce inflammation. In this study, ADA activity increased dramatically in the PA-intoxicated group. We speculate that, the aforementioned energy-sensing function of adenosine may a reason for this. The concentration of palmitic acid used in the present study (5 mM) is far more than the physiological level (0.5 mM), suggesting that, while the body metabolizes the required PA, the excess accumulates in the brain, and causes surplus calories. The excessive calorie then increases the brain adenosine level that necessitates increased catabolism by ADA (Antonioli *et al.*, 2012). Avci and Durak (2008) suggested that increased ADA activity correlates positively with pathological conditions linked to a deranged immune system and inflammation. To our knowledge, we are reporting for the first time, the involvement of ADA in PA-induced neuroinflammation in rats. Nevertheless, the activity of ADA was normalized in the lycopene supplemented group, further affirming the therapeutic effect of lycopene against neuro-inflammatory triggers and mediators (Kuhad et al., 2008). Considering the putative role of ADA in inflammatory diseases, the anti-inflammatory effects of lycopene might be responsible for the observed reduction of ADA activity in the lycopene- treated groups (Avci and Durak, 2008).

Akin to AchE and ADA, monoamine oxidase-A (MAO-A) is involved in neurologic disorders (Akintunde et al., 2018). Monoamine oxidases (MAOs) are a group of Flavin-containing enzymes that cleaves the amino group from biogenic and xenobiotic amines in vivo and functions in, the mood control, motivation and reward in the brain (Nwanna et al., 2019). Thus, the elevated activity of MAO-A in this study suggests that, habitual consumption PA- rich diet might trigger a cascade of cognition deficit-inducing condition. Saada et al. (2010) reported an increase in the MAO activity following exposure of rats to gamma radiation. Suggesting that monoamine hyper-activation might accompany inflammation. We, however, observed a remarkable downregulation of this enzyme activity in the lycopene supplemented groups. Our observation is consistent with that of El-Morsy and Ahmed (2020), who suggested that the ability of

lycopene to diminish cognitive deficit might be due to attenuation of oxidative stress and upregulation of BDNF.

The activity of ectonucleotidases (NTPDases) usually shows the extent of nucleotide breakdown in the cell (ATP hydrolysis), and might be used as an index to assess the bioenergetic status of the brain (Akintunde et al., 2018). ATPase breaks down ATP into ADP and Pi with a concomitant release of energy for the metabolic process while 5'-Nucleotidase (5'-NTD) breaks down AMP to adenosine and Pi. The cumulative effect of these enzymatic reactions is a wholesale reduction in brain nucleotide and energy levels (Belló-Klein *et al*., 2016). In our study, NTPDase and 5'-NTD activities increased significantly in the PA-treated groups suggesting a reduced brain nucleotide pool and energy shortage, impaired purinergic signalling, necrosis, and possible cell death (Li et al., 2015). Furthermore, generation of ROS due to excessive PA metabolism might induce mitochondrial dysfunction and uncouple energy generation leading to neural apoptosis and cognition impairment (Massaad and Klann, 2011). Our data showed that lycopene reversed these purinergic enzymes abnormalities suggesting an increase in ATP levels necessary for proper brain functioning. These data portend beneficial attributes possessed by lycopene in the management of impaired neuro-behavioural enzymes due to PA intoxication in the central nervous system. The observation is consistent with the study of Malekiyan et al. (2019), where lycopene attenuated neuronal damages induced by diabetes in rats by improving the purinergic enzyme activities.

The causal role of oxidative stress in neurodegenerative disorders is undisputable. Perhaps, due to the large oxidative-species-generating capacity of the brain, its limited antioxidative ability, and abundance of polyunsaturated fatty acids, the brain cells are susceptible to oxidative damage (Salim, 2017). Oxidative stress is driven by ROS when the cells' ability to neutralize them is overwhelmed, or when the antioxidant system (including enzymes such as catalase, and glutathione peroxidase) is impaired (Calabrese *et al*., 2008). Our data shows that PA causes oxidative stress by significantly lowering the catalase and glutathione peroxidase activities (Fig. 3). PA also caused a copious increase in the MDA level (an index of lipid peroxidation) coupled with abatement in GSH level in the brain of the untreated PA-challenged rats. The inhibition of catalase and glutathione peroxidase specific activities, following the PA challenge is consistent with the observations of Alnahdi et al. (2019) who reported that high PA and glucose caused significant reduction of the antioxidant enzymes and caused upregulation of inflammatory cytokines. Further, the activity of myeloperoxidase was evaluated in the brain to substantiate other sources of ROS and inflammation following PA administration. Myeloperoxidase (MPO) is produced by polymorphonuclear cells with pro-inflammatory and oxidative functions. It is secreted by activated monocytes, microglia, and macrophages as a direct response to oxidative stress, pathogen-associated molecular patterns (PAMPS) or damage-associated molecular patterns (DAMPS) bindings. It also increases the production of the pro-inflammatory cytokines (Nussbaum et al., 2013; Kim *et al*., 2019). The MPO in conjugation with hydrogen peroxide might cause the elevation of MDA and activate inflammation via the up-regulation of inducible nitric oxide synthase (iNOS) (Galijasevic *et al*., 2003; Kim *et al*., 2019). Upregulation of MPO following the PA challenge in this study lends more credence to its ability to elicit cellular dysfunction when in excess. Nevertheless, lycopene administration essentially abated the occurrence of oxidative stress by normalizing the activities of CAT, GPX, and MPO as well as augmenting

the level of GSH in the treated groups. In the same vein, the MDA level decreased meaningfully in the lycopene-treated rats when compared with the untreated group. Consistent with earlier reports on the attenuation of oxidative stress indices in the hippocampus of diabetic rats (Malekiyan et al., 2019), our observations further substantiate the antioxidant effect of lycopene and its ability to inhibit lipid peroxidation, break the chain of ROS production, and induce antioxidant enzymes (Malekiyan et al., 2019; El-Morsy and Ahmed, 2020).

Excessive PA has putative detrimental effects on whole-body metabolism, especially de novo lipogenesis (DNL) of regulatory substances such as palmitoylethanolamide (PEA) and dipalmitoyl-phosphatidylcholine (DPPC) (Carta *et al*., 2017). Disproportionate energy balance might disrupt the tightly controlled regulation of tissue PA, thus leading to dyslipidemia, hyperglycemia, and ectopic fat accumulation, which could activate the inflammatory TLR4/TAK1/IKK/NF-κB-p65 pathways (Carta *et al*., 2017). Indeed, disruption of PA homeostatic balance has been implicated in atherosclerosis, cancer, and neurodegenerative disease (Innis, 2016; Carta *et al*., 2017). We measured some brain lipids levels to ascertain if PA could disrupt its metabolism as part of /or an alternative mechanism of PA-mediated neuroinflammation. Our data revealed a significant reduction in the brain phospholipids and cholesterol contents with a concomitant increase in TAG level in the PA untreated group (Fig. 4). This trend suggests that PA might cause an imbalance in lipid metabolism in the brain. The mechanism behind these observations is unclear. However, de novo lipogenesis (DNL) and exogenous PA are reciprocally regulated to maintain a balanced saturated/unsaturated fatty acid ratio (SAFA/UFA). Excessive PA are desaturated and incorporated into the cell membrane. The desaturation process is necessary to maintain the concentration of phospholipids in a physiological range. However, increased PA level distorts the SAFA/UFA ratio in-favour of SAFA, causing a reduction in the phospholipids contents (Abbott et al., 2012). This disturbance might be responsible for the lowering of phospholipids levels observed in this study. Furthermore, imbalance of SAFA/UFA may invoke the transcription factors responsible for cholesterol and TAG biosynthesis. The net result is an increased TAG and cholesterol production. As observed in this study, TAG level increased in the PA-untreated group, and TAG elevation is prodromal to inflammatory responses (Carta *et al*., 2017), however, cholesterol level decreased. Lycopene has been shown to possess the ability to reduce intracellular cholesterol own to its hypo-cholesterolemic effect, thereby, inhibiting an aberrant cholesterol accumulation in the cell (Palozza et al., 2012).

Neuroinflammation is driven by the master regulator of inflammation – NF-κB (Dalvi *et al*., 2017) via the toll-like receptor- 4 (TLR4) and culminates into secretion of pro-inflammatory cytokines such as interleukin1-beta (IL-1β), interleukin 6 (IL-6), and tumour necrosis factor-alpha (TNF-α) (Dali-Youcef, and Ricci, 2015). Alternatively, the inflammatory cue might invoke the leucine-rich repeats containing pyrin domain 3 (NLRP3) inflammasome activation via the IL-1beta receptor (Dali-Youcef, and Ricci, 2015). The endpoint of these activation leads to endoplasmic reticulum stress and attendant inflammatory responses. In this study, upregulation of the NF-κB -p65, parallels with concomitant increments in the expression of gene targets such as IL-1β, and IL-6 (Fig. 5). This suggests that PA-induced the activation of the NF-kB/TLR4/MyDD88 signalling cascade leading to the production of the pro-inflammatory cytokines. Our observations are consistent with other studies (Tse et al., 2018; Alnahdi et al., 2019).

Besides, upregulation of IL-1β in the CNS during HFD feeding might contribute to neuroinflammation and blood-brain-barrier (BBB) disruption (Jais and Bruning, 2017). IL-10 relative expression, on the other hand decrease in the PA control group, suggesting that PA overload might decrease the expression of antiinflammatory cytokines, which may aggravate neuronal damage (Steen et al., 2020). Nevertheless, our results showed normalization of IL-10 level in the lycopene-treated groups suggesting its modulatory effects on aberrant production of cytokines. Regardless of perturbations imposed on the inflammatory pathways by PA, lycopene essentially and significantly abated NF-κB -p65 inflammatory response in the groups treated with lycopene, especially the 0.48 mg/kg dose. This observation did not only authenticate the anti-inflammatory effect of lycopene but its neuroprotective effects (Kuhad et al., 2008; Malekiyan *et al*., 2018) perhaps due to its lipophilicity and ability to easily cross the blood-brain barrier to modulate cellular processes.

Disruption of biochemical processes often results in histological impairments, and, this represents the basis for tissue damage and loss of function (Greaves and Faccini, 2007). In the present study, the PA group showed severe vacuolation of the brain (Fig. 6). Vacuolation of the brain cytoplasm is a degenerative feature arising from xenobiotic insult (Greaves and Faccini, 2007), and is mostly caused by damaged myelin sheath in the brain, suggesting that PA could initiate a complex yet unresolved process that causes demyelination of the CNS. The root mechanism(s) behind the vacuolar bodies formation in the cell might be a result of endoplasmic reticulum (ER) stress (Lee *et al*., 2019). Oxidative stress might invoke ER stress by causing protein misfolding which activates the unfolded protein response (UPR). Indeed, this is another mechanism behind PA-mediated activation of the NLRP3 inflammasome activation (Dali-Youcef, and Ricci, 2015). Groups treated with lycopene showed normal brain architecture when compared with the untreated group, thereby substantiating the neuroprotective effects of lycopene against PA-induced neuroinflammation.

Conclusion

Conclusively, excessive PA intake caused the alteration of neuro-behavioural enzymes activities and induced oxidative stress by inhibiting the antioxidant system. Furthermore, it also caused dysregulation in lipid metabolism, as well as upregulation of the mRNA levels of pro-inflammatory cytokines. Nevertheless, lycopene showed tremendous ameliorative effects a PA-induced neuroinflammation in rats. This is characterized by normalization of neurobehavioral enzymes, attenuation of oxidative stress indices, modulation of lipid metabolism, and abatement of inflammatory cytokines. The underlying mechanism might be through the attenuation of oxidative stress and downregulation of TLR4/NF-κB -p65 inflammatory axis

Declarations

Conflict of Interests

We declare no known conflict of interest

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References

1. Abbott, S. K., Else, P. L., Atkins, T. A. & Hulbert, A. J. Fatty acid composition of membrane bilayers: importance of diet polyunsaturated fat balance. *Biochim Biophys Acta Biomembr.* **1818**, 1309–1317 (2012).
2. Akintunde, J. K., Irondi, A. E., Ajani, E. O. & Olayemi, T. V. Neuroprotective effect of dietary black seed flour on key enzymes linked with neuronal signaling molecules in rats exposed to mixture of environmental metals. *J. Food Biochem.* **42**, e12573 (2018).
3. Akinyemi, A. J. *et al.* Effect of dietary supplementation of ginger and turmeric rhizomes on ectonucleotidases, adenosine deaminase and acetylcholinesterase activities in synaptosomes from the cerebral cortex of hypertensive rats. *J. Appl. Biomed.* **14**, 59–70 (2016).
4. Alnahdi, A., John, A. & Raza, H. 2019 Augmentation of Glucotoxicity, Oxidative Stress, Apoptosis and Mitochondrial Dysfunction in HepG2 Cells by Palmitic Acid. Nutrients.11, 1979–1986.
5. Antonioli, L. *et al.* M., 2012 Adenosine deaminase in the modulation of immune system and its potential as a novel target for treatment of inflammatory disorders.Curr. Drug Targets13,842–62.
6. Avci, A. & Durak, I. 2008 Tomato Juice, Prostate Cancer and Adenosine Deaminase Enzyme. *Tomatoes and Tomato Products: Nutr Med Ther Prop*.457.
7. Beuge, J. A. & Aust, S. D. 1978 Estimation of serum malondialdehyde level. *Methods in enzymology Hoffee Jones edt. By Hoffee PA and Jone ME. Academic Press, a Subsidinary of Harcoart Brace Jovanovich Publisher, New York.*
8. Bala, R., Khanna, D., Mehan, S. & Kalra, S. Experimental evidence for the potential of lycopene in the management of scopolamine induced amnesia. *RSC advances.* **5** (89), 72881–72892 (2015).
9. Calabrese, V. *et al.* 2008 Cellular stress response: a novel target for chemoprevention and nutritional neuroprotection in aging, neurodegenerative disorders and longevity.Neurochem. Res.33,2444–71.
10. Camandola, S. & Mattson, M. P. Brain metabolism in health, aging, and neurodegeneration. *The EMBO J.* **36**, 1474–1492 (2017).
11. Card, J. W. *et al.* Gender differences in murine airway responsiveness and lipopolysaccharide-induced inflammation. *J Immunol.* **177**, 621–630 (2006).
12. Carta, G., Murru, E., Banni, S. & Manca, C. 2017 Palmitic acid: physiological role, metabolism and nutritional implications.Front. Physiol8,902.
13. Cavalcante, S. F. *et al.* 2020 Acetylcholinesterase: The “Hub” for Neurodegenerative Diseases and Chemical Weapons Convention.Biomolecules. 414,1–10.

14. Chen, D., Huang, C. & Chen, Z. 2019 A review for the pharmacological effect of lycopene in central nervous system disorders.Biomed. Pharmacother111,791–801.
15. Cristalli, G. *et al.* 2001 Adenosine deaminase: functional implications and different classes of inhibitors.Med. Res. Rev. 2,105–28.
16. Dali-Youcef, N. & Ricci, R. 2015 Signalling networks governing metabolic inflammation. In Metabolic Control Springer, **Cham**. **pp**. 195–220.
17. Dalvi, P. S. *et al.* 2017 High fat induces acute and chronic inflammation in the hypothalamus: effect of high-fat diet, palmitate and TNF-α on appetite-regulating NPY neurons.Int J Obes. 41,149–58.
18. Dangana, E. O., Michael, O. S., Omolekulo, T. E., Areola, E. D. & Olatunji, L. A. Enhanced hepatic glycogen synthesis and suppressed adenosine deaminase activity by lithium attenuates hepatic triglyceride accumulation in nicotine-exposed rats. *Biomed. Pharmacother.* **109**, 1417–1427 (2019).
19. Devaraj, S. *et al.* 2008 A dose-response study on the effects of purified lycopene supplementation on biomarkers of oxidative stress.J Am Coll Nutr.*27*,267–273.
20. El Morsy, E. M. & Ahmed, M. A. Protective effects of lycopene on hippocampal neurotoxicity and memory impairment induced by bisphenol-A in rats. *Hum Exp Toxicol.* **10**, 0960327120909882 (2020).
21. El-Aaser, A. B. & El-Merzabani, M. M. Simultaneous determination of 5'-nucleotidase and alkaline phosphatase activities in serum. *Z. Klin. Chem. Klin. Biochem.* **13**, 453–459 (1975).
22. Ellman, M. 1959 A spectrophotometric method for determination of reduced glutathione In tissues.Ann. Biochem.*74*,214–26.
23. Gaber, T., Strehl, C. & Buttgereit, F. 2017 metabolic regulation of inflammation.*Nat Rev Rheumatol*.13,267–279. https://doi.org/10.1038/nrrheum.2017.37.
24. Galijasevic, S., Saed, G. M., Diamond, M. P. & Abu-Soud, H. M. 2003 Myeloperoxidase up-regulates the catalytic activity of inducible nitric oxide synthase by preventing nitric oxide feedback inhibition. **PNAS**. 100, 14766-71.
25. Greaves, P. & Faccini, J. M. *Nervous system and special sense organs: Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Evaluation, Third Edition*861–933(Elsevier Inc., Oxford, UK., 2007).
26. Hanamsagar, R. & Bilbo, S. D. Sex differences in neurodevelopmental and neurodegenerative disorders: focus on microglial function and neuroinflammation during development. *J. Steroid Biochem. Mol. Biol.* **160**, 127–133 (2016).
27. Innis, S. M. Palmitic acid in early human development. *Crit Rev Food Sci Nutr.* **56**, 1952–1959 (2016).
28. Jais, A. & Brüning, J. C. Hypothalamic inflammation in obesity and metabolic disease. *J Clin Invest.* **127**, 24–32 (2017).
29. Katewa, S. D. & Katyare, S. S. A simplified method for inorganic phosphate determination and its application for phosphate analysis in enzyme assays. *Analy.* **Biochem. 323**, 180–187 (2003).

30. Kim, H. J. *et al.* 2019 Reducing myeloperoxidase activity decreases inflammation and increases cellular protection in ischemic stroke. **J Cerebr Blood F Met**. 39,1864–77.
31. Klebanoff, S. J., Waltersdorph, A. M. & Rosen, H. 1984 [52] Antimicrobial activity of myeloperoxidase.**Meth. Enzymol.**105,399–403.
32. Kozicz, T. & Morava, E. 2018 A new opportunity: metabolism and neuropsychiatric disorders.*J Inherit Metab Dis.***41**,583–584https://doi.org/10.1007/s10545-018-0215-y.
33. Kuhad, A., Sethi, R. & Chopra, K. Lycopene attenuates diabetes-associated cognitive decline in rats. *Life Sci.* **83**, 128–134 (2008).
34. Lee, W. J. *et al.* 2015 Nonautophagic cytoplasmic vacuolation death induction in human PC-3M prostate cancer by curcumin through reactive oxygen species-mediated endoplasmic reticulum stress. Sci. Rep 5, 10420.
35. Leng, Y., Musiek, E. S., Hu, K., Cappuccio, F. P. & Yaffe, K. 2019 Association between circadian rhythms and neurodegenerative diseases. **Lancet Neurol**.18, 307 – 18.
36. Li, Q. *et al.* The preferential accumulation of heavy metals in different tissues following frequent respiratory exposure to PM 2.5 in rats. *Sci. Rep.* **5**, 1–8 (2015).
37. Malekiyan, R., Abdanipour, A., Sohrabi, D. & Jafari, A. I. Antioxidant and neuroprotective effects of lycopene and insulin in the hippocampus of streptozotocininduced diabetic rats. *Biomed. Rep.* **10**, 47–54 (2019).
38. Marklund, S. & Marklund, G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem.* **47**, 469–474 (1974).
39. Massaad, C. A. & Klann, E. 2011 Reactive oxygen species in the regulation of synaptic plasticity and memory.Antioxid Redox Signal.14, 2013–2054.
40. Mattson, M. P. Pathways towards and away from Alzheimer's disease. *Nature.* **430**, 631–639 (2004).
41. Mattson, M. P. Interventions that improve body and brain bioenergetics for Parkinson's disease risk reduction and therapy. *J Parkinsons Dis.* **4**, 1–13 (2014).
42. Nussbaum, C., Klinke, A., Adam, M. & Baldus, S. Myeloperoxidase: a leukocyte-derived protagonist of inflammation and cardiovascular disease. *Antioxid Redox Signal.* **18**, 692–713 (2013).
43. Nwanna, E. E., Adebayo, A. A., Oboh, G., Ogunsuyi, O. B. & Ademosun, A. O. Modulatory effects of alkaloid extract from Gongronema latifolium (Utazi) and Lasianthera africana (Editan) on activities of enzymes relevant to neurodegeneration. *J. Diet. Suppl.* **16**, 27–39 (2019).
44. Palozza, P. A. O. L. A., Catalano, A., Simone, R. E., Mele, M. C. & Cittadini, A. Effect of lycopene and tomato products on cholesterol metabolism. *Ann Nutr Met.* **61**, 126–134 (2012).
45. Rao, A. V. & Rao, L. G. Carotenoids and human health. *Pharmacol Res.* **55**, 207–216 (2007).
46. Rotruck, J. T. *et al.* 1973 Selenium: biochemical role as a component of glutathione peroxidase.Science. 179,588–90.
47. Sahin, K. *et al.* 2014 Orally administered lycopene attenuates diethylnitrosamine-induced hepatocarcinogenesis in rats by modulating Nrf-2/HO-1 and Akt/mTOR pathways. **Nutr Cancer**. 66,

590-8.

48. Salim, S. 2017 Oxidative stress and the central nervous system.J Pharmacol Exp Ther. *360*,201–205.
49. Schetinger, M. R. C., Morsch, V. M., Bonan, C. D. & Wyse, A. T. 2007 NTPDase and 5'-nucleotidase activities in physiological and disease conditions: New perspectives for human health.*Biofactors*. *31*,77–98.
50. Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. 2012 NIH Image to ImageJ: 25 years of image analysis.Nat.Methods.9,671–5.
51. Shangari, N. & O'Brien, P. J. Catalase activity assays. *Curr Protoc toxicol.* **27**, 1–7 (2006).
52. Sherwin, C. M. *et al.* 2003 Guidelines for the ethical use of animals in applied ethology studies.Anim. Behav. Sci.81,291–305.
53. Steen, E. H. *et al.* 2020 the role of the anti-inflammatory cytokine interleukin-10 in tissue fibrosis.**Adv** *Wound Care*. 9,184–98.
54. Tangvarasittichai, S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World J. Diabetes.* **6**, 456 (2015).
55. Tashiro, H. *et al.* 2017 Saturated Fatty Acid Increases Lung Macrophages and Augments House Dust Mite-Induced Airway Inflammation in Mice Fed with High-Fat Diet. Inflammation *40*, 1072–1086. https://doi.org/10.1007/s10753-017-0550-4.
56. Tilg, H., Zmora, N., Adolph, T. E. & Elinav, E. 2020 The Intestinal Microbiota fuelling metabolic inflammation.**Nat. Rev. Immuno**.20,40–54
57. Tomassoni, D. *et al.* 2020 Obesity and Age-Related Changes in the Brain of the Zucker Lepr fa/fa Rats. **Nutrients**.12, 1356.
58. Tse, E. K., Salehi, A., Clemenzi, M. N. & Belsham, D. D. Role of the saturated fatty acid palmitate in the interconnected hypothalamic control of energy homeostasis and biological rhythms. *Am J Physiol-Endoc.* **315**, 133–140 (2018).
59. Ugbaja, R. N. *et al.* 2016 Ameliorative Effects of Aloe Vera Gel Extract on Circulatory and Tissue Lipid Profile Status in Streptozotocin-Induced Diabetic Rats. *Bangladesh* **J. med. biochem**. 9,11–26.
60. Umuerri, E. M., Ayandele, C. O. & Eze, G. U. Prevalence and sociodemographic correlates of obesity and overweight in a rural and urban community of Delta State, Nigeria. *Sahel Med J.* **20**, 173 (2017).
61. Wang, Y. *et al.* 2017 Saturated palmitic acid induces myocardial inflammatory injuries through direct binding to TLR4 accessory protein MD2.Nat. Commun. *8*,1–13. https://doi.org/10.1038/ncomms13997
62. Wang, Z., Fan, J. & Wang, J. Protective effect of lycopene on high-fat diet-induced cognitive impairment in rats. *Neurosci Lett.* **627**, 185–191 (2016).
63. Yu, L. *et al.* 2017 Dietary lycopene supplementation improves cognitive performances in tau transgenic mice expressing P301L mutation via inhibiting oxidative stress and tau hyperphosphorylation.J Alzheimers Dis. 57,475–82.