

The Effects of *Abelmoschus esculentus* (L.) Moench Seed on High Fat Diet Induced Metabolic and Cognitive Impairments in C57BL/6J Mice

(Kesan Biji *Abelmoschus esculentus* (L.) Moench pada Kerosotan Metabolik dan Kognitif Terinduksi Diet Tinggi Lemak dalam Tikus C57BL/6J)

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ABSTRACT

Okra is known for its neuroprotective and antioxidant properties. We aimed to investigate the potential effects of okra seed powder in alleviating high fat diet HFD-induced cognitive deficit and hypercholesterolemia. We randomly allocated thirty-six C57BL/6J male mice into: (i) control, mice fed with a normal fat level diet; (ii) HFD, mice fed with HFD; (iii) HFD-OS1; (iv) HFD-OS2; (v) HFD-OS3, mice fed with HFD and okra seed powder (200, 400, or 800 mg/kg/day, respectively); (vi) HFD-SIM, mice fed with HFD and simvastatin (20 mg/kg/day). After 10 weeks of treatment period, the mice were tested with an episodic-like memory test (EMT) and Morris water maze (MWM). We found significantly higher total and LDL cholesterol levels in mice fed with HFD. Compared to the HFD group, the control group performed better in the EMT test, and also learned and retrieved spatial reference memory better in the MWM test. The okra seed powder significantly improved spatial learning in four days of acquisition trials and the highest dose of okra profoundly improved spatial reference memory retention during the probe trial. Contrary to the MWM results, the okra-treated animals did not perform significantly better than the HFD-treated animals in EMT. At present, we recommend future studies testing the potential neuroprotective or cognitive enhancing effects of okra to assess different cognitive domains using various disease models to have a better understanding on the potential neuroprotective properties of okra.

Keywords: *Abelmoschus esculentus* (L.) Moench (okra); cognitive impairment; high-fat diet

ABSTRAK

Okra terkenal dengan kesan antioksidan dan neuropelindungannya. Kajian ini bertujuan untuk menilai sama ada serbuk biji bendi mampu mengurangkan kerosakan kognitif dan hiperkolesterolemia akibat HFD. Kami secara rawak membahagikan tiga puluh enam mencit jantan C57BL/6J kepada: (i) kawalan, mencit yang diberi makan dengan diet yang mengandungi tahap lemak normal; (ii) HFD, mencit diberi makan dengan HFD; (iii) HFD-SIM, mencit diberi makan dengan HFD dan diberikan simvastatin (20 mg/kg/hari); (iv) HFD-OS1; (v) HFD-OS2; (vi) HFD-OS3, mencit yang diberi HFD dan serbuk biji bendi (masing-masing 200, 400, atau 800 mg/kg/hari). Setelah 10 minggu rawatan, mencit telah didedahkan kepada ujian ingatan seperti episodik (EMT) dan *Morris Water Maze* (MWM). Mencit yang diberi makan dengan HFD mencatatkan jumlah dan tahap kolesterol LDL yang jauh lebih tinggi. Untuk EMT, haiwan kawalan berprestasi lebih baik daripada kumpulan HFD. Dalam ujian MWM, haiwan kawalan belajar dan mendapatkan semula ingatan rujukan reruang dengan lebih ketara berbanding kumpulan HFD. Serbuk biji bendi tidak menghasilkan

sebarang kesan ketara dalam EMT, walau bagaimanapun, pembelajaran ruwang bertambah baik dengan ketara dalam empat hari percubaan pemerolehan dan dos tertinggi bendi meningkatkan pengekaln memori rujukan ruwang dengan ketara semasa *Probe Trial*. Walaupun dengan ketara meningkatkan pembelajaran ruwang dan pengekaln ingatan dalam MWM, tiada haiwan yang dirawat okra menunjukkan prestasi yang lebih baik daripada kumpulan HFD dalam EMT. Oleh itu, kami mengesyorkan kajian masa depan yang berkaitan dengan okra untuk menguji komponen kognitif yang berbeza untuk mempunyai pemahaman yang jelas tentang kesan neuropelindung okra.

Kata kunci: *Abelmoschus esculentus* (L.) Moench (okra); diet tinggi lemak; kemerosotan kognitif

INTRODUCTION

Based on 2016 data by the World Health Organization, around 13% of world's adult population were obese, and the mortality rate of obesity and its complications affect 2.8 million people worldwide (WHO 2018). Moreover, obesity is associated with metabolic syndrome, a group of metabolic disorders including hypertension, glucose intolerance or insulin resistance, elevated prothrombotic state or proinflammatory state, atherogenic dyslipidemia, and visceral adiposity (Huang 2009).

Deterioration of brain function and physiology are considered as one of the clinical complications of obesity. Temporal lobe, frontal lobe, occipital lobe, and hippocampal atrophy were detected in the brain of obese patients (Shefer, Marcus & Stern 2013). Obesity and insulin resistance also are associated with cognitive impairment as measured by episodic memory tasks (Cheke et al. 2017). A cohort study in Australia showed that adherence to a healthy diet reduces the risk of cognitive disruption (Gardener et al. 2015). At preclinical level, high fat diet (HFD)-induced cognitive detriment is well established in mice, where even biochemical changes such as neurofibrillary tangle formation and amyloid beta deposition, which are common to Alzheimer's disease was reported (Kothari et al. 2017). Major proposed mechanisms linking obesity are altered blood brain barrier integrity, inflammation, insulin resistance, and oxidative stress (Cordner & Tamashiro 2015; Nurul 'Ain, Teoh & Mohamad Fairuz 2018).

Oxidative stress is the underlying cause of many diseases (Amilia et al. 2020; Kumar et al. 2017), including neuroinflammation and brain dysfunction (Kamal et al. 2020). Consumption of functional food, especially natural products has been on the rise as a practice of alternative medicine due to their various medicinal properties (Adila et al. 2020; Chua et al. 2021; Kamal et al. 2021; Khidhir et al. 2020, 2018). *Abelmoschus esculentus* (L.) Moench, also known as lady's finger or

okra, exhibit a wide range of medicinal properties such as anti-inflammation, anti-dyspeptic, anti-diabetic, diuretic, anti-oxidant and laxative (Kaewsrichan, Wongwitwichot & Manee 2020; Manee & Kaewsrichan 2017; Ngog et al. 2008; Roy, Shrivastava & Mandal 2014). Okra seeds contain a broad range of compounds, including tannins, glutathione, saponins, terpenoids, flavonoids, long chain fatty acids, polyphenols, sterols and their derivatives (Kaewsrichan, Wongwitwichot & Manee 2020). Hence, due to its overwhelming medicinal properties, okra seed, has garnered much attention as a potential therapeutic agent in prevention of various diseases. Therefore, we aimed to explore the effects of okra seed in attenuation of HFD-induced hyperlipidemia and cognitive impairment. Previously, we have reported okra peel to significantly improve the learning ability and memory retention of mice fed with HFD (Prom-In et al. 2020).

MATERIALS AND METHODS

PREPARATION OF OKRA SEED POWDER

Fresh okra pods were obtained from a local market in Hat-yai, Songkhla province, Thailand. Okra seeds were collected and dried in a hot air oven at 60 °C. The dried seeds, then, were ground and sieved out to the 1-mm mesh. The fine powder of okra seed was kept in an air-tight container at room temperature until use.

ANIMALS

Six-weeks-old male C57BL/6J mice (Monash University Malaysia Sdn Bhd), weighing 16-20 g were used in this study. The mice were kept in a room maintained on a 12-h light/dark cycle and at 22±2 °C with free access to water and diet. All experimental procedures were conducted in accordance with Universiti Kebangsaan Malaysia Guidelines for the Care and Use of Laboratory Animals (approval number: FISIO/PP/2018/JAYAKUMAR/26-SEPT./955-OCT.-2018-MAC.-2019).

ANIMAL TREATMENT

The mice were randomly allocated into 6 groups ($n=6$ in each group). All animal diets were purchased from Altromin Spezialfutter GmbH & Co. (Germany) with its content shown in Table 1. During 14 days of acclimatisation period, all animals were first fed with a cereal-based diet (Altromin 3114), and then with the control diet with normal fat content (Altromin C1090-10). The animals belonging to the control group were fed with the control diet for 12 weeks. The rest of the five treatment groups were fed with the HFD (HFD; Altromin C1090-60) alone, HFD supplemented with 200 mg/kg/day okra seed (HFD-OS1), HFD supplemented with 400 mg/kg/day okra seed (HFD-OS2), HFD supplemented with 800 mg/kg/day okra seed (HFD-OS3) and HFD supplemented with 20 mg/kg/day simvastatin as a treatment control (HFD-SIM). The mice' body weights and food intake were recorded daily. After 12 weeks, the animals were euthanised with 30 mg/kg pentobarbital, and their serum samples were collected, separated by centrifugation ($2000\times g$) for 15 minutes and stored at $-30\text{ }^{\circ}\text{C}$.

LIPID PROFILE ASSESSMENT

The mice sera were sent to the Pathology and Clinical Laboratory (M) Sdn. Bhd., Kuala Lumpur, Malaysia for serum total cholesterol, triglyceride, and low-density lipoprotein (LDL) assessments. Assessment was carried out using SIEMENS ADVIA® Chemistry system based on SIEMENS Healthcare Diagnostics Inc. instructions.

EPISODIC-LIKE MEMORY TEST

The test was performed as previously described (Dere, Huston & De Souza Silva 2005a). The long-term memory for spatial location of objects, order of presentation and types of objects were assessed using an open field ($25 \times 25\text{ cm}$). In brief, 10 days prior to the experiment, each animal underwent a pre-training session for familiarising with the test apparatus and the exploration task. Then, each mouse was subjected to two sample trials and a discrimination trial, in which the objects and locations were altered as indicated by Figure 1. The tests were carried out for 10 min and the between-trial resting time was set at 50 min, and the object exploration time was recorded. The exploration behaviour was assumed when the animals approached and had physical contact with the objects, either with snout, forepaws or vibrissae. The animal behaviour was recorded using Logitech HD webcam C310 and Smart software (version 3.0.06, Panlab).

MORRIS WATER MAZE (MWM) TEST

Evaluation of spatial learning and memory retention was carried out through the MWM test based on (Bromley-Brits, Deng & Song 2011; Prom-In et al. 2020). A circular pool (40 cm height and 100 cm in diameter) was filled with water (depth 30 cm) and the water temperature was maintained at $25\pm 2\text{ }^{\circ}\text{C}$ in the center of a homogeneously lit (3 Lux) soundproof room. The MWM was divided into four equal quadrants. To record the animals' activities in the MWM, a video camera was placed perpendicular to the center of the water maze, to the ceiling. A square

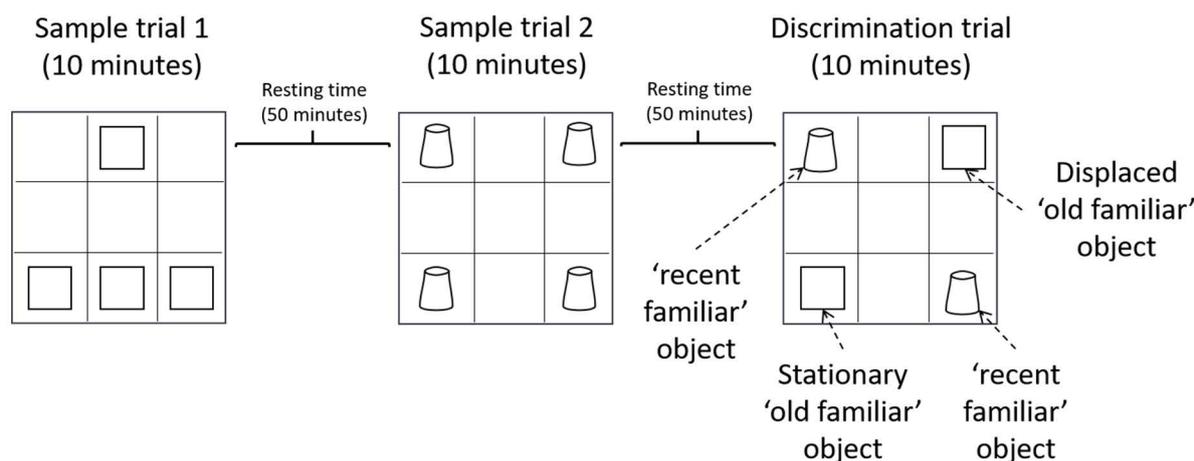


FIGURE 1. The experimental scheme of episodic-like memory test

white platform (3 × 3 inches) was submerged 1 inch below the water surface during pre-training session, raised 1 inch above the water surface during the acquisition trial, and completely removed from the pool during the probe trial. A visual clue was placed on the north wall of the experiment room throughout the experiments. On the first training day, all animals were trained to search for a randomly located visible platform for 5 sessions. Each session was 1 min with a 5-min trial interval, and each trial started from a different location. For the next 4 consecutive days of acquisition trials, the mice were allowed to look for the submerged platform for 5 sessions per day. The final probe trial was performed on the 6th day, 24 h from the last acquisition trial on day 5. The time and distance animals used in a swimming arena to locate the platform was recorded and acquired by using Logitech HD webcam C310 and Smart software (version 3.0.06, Panlab), respectively.

STATISTICAL ANALYSIS

Kolmogorov–Smirnov test was performed to verify the normal distribution of the data (each $p > 0.05$). All data were expressed as means ± SEM. Generally, one-way ANOVA was used to analyze data such as food intake, serum lipid profile, calorie consumption, average speed (MWM), entries in platform zone and time spent in the platform zone during the probe trials. Data generated from body weight changes and acquisition trials were evaluated using repeated measure of ANOVA and post hoc Tukey test using the SPSS statistical software package version 19 (IBM Corporation, Armonk, NY, USA). For variables with unequal variances among groups, the significance of differences between the groups was analyzed using the Welch test with Dunnett T3 post hoc test was used to determine the pairwise differences. Data were expressed as the mean ± standard error of the mean (S.E.M). Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

BODY WEIGHT CHANGES AND LIPID PROFILE

Total body weight of all groups increased gradually throughout the experiment (Figure 2), and in general, the mice fed with HFD weighed higher than the control group. The results were in accordance with serum lipid profile, in which the total cholesterol and LDL levels of animals that received HFD were significantly higher than the control group (Table 2). Body weight of the control groups was well maintained (30% increased from initial weight), which is in accordance with the amount of calorie intake (Table 2). Although the amount of food intake by the control group was significantly higher than other experimental groups (Table 2), the calorie intake was

significantly lower. The body weight of animals treated with simvastatin was within the similar range of the control group for the first five weeks. For the remaining seven weeks of treatment period, the simvastatin-treated animals weighed more than the control group, and at the same time, weighed lesser than the rest of the HFD-fed groups. Treatment with simvastatin did not cause significant changes in the lipid profile of HFD group. Additionally, we did not find any significant effect of OS on the lipid profile and body weight of HFD group.

EPISODIC-LIKE MEMORY TEST

The control and HFD-OS3 ($p < 0.05$) groups spent profoundly more time exploring ‘old familiar’ objects than ‘recent familiar’ objects (one-tailed t-test for dependent groups) (Figure 3). A two-tailed single group t-test showed that the control animals ($p < 0.05$) significantly preferred the ‘old familiar’ objects, however, the preference ratio of the HFD-OS3 group for ‘old familiar’ objects were not statistically significant. In a separate observation, the control, HFD-OS2, and HFD-OS3 groups spent more time exploring the non-displaced ‘old familiar’ objects, however, the findings were not significant ($p > 0.05$). The control animals also spent markedly more time exploring the displaced ‘old familiar’ objects compared to the stationary ‘old familiar’ objects ($p < 0.05$), however the assessment of preference ratio (for displaced old familiar objects) against chance (0.5) was not statistically significant.

MWM TEST

We did not find any significant difference in the path length during acquisition trials between the groups (Day 1-4, $p > 0.05$) (Figure 4) However, we found a significant difference between the control and HFD groups in their total path length throughout the acquisition trials ($p < 0.05$). We also noticed a profound difference between the groups on Day 2 ($p < 0.05$), especially between the HFD and control ($p < 0.001$), HFD-OS1 ($p < 0.001$), HFD-OS2 ($p < 0.001$), HFD-OS3 groups ($p < 0.001$), and HFD-SIM ($p < 0.01$) in escape latency during the acquisition trials. As for the total escape latency (D1-D4 of acquisition trials), we found profound differences between the HFD and control, HFD-OS1, HFD-OS2, and HFD-OS3 groups ($p < 0.01$). Compared to the HFD group, other treatment groups spent more time in the platform zone on day 2 (control, $p < 0.01$; HFD-OS1, $p < 0.01$; HFD-OS2, $p < 0.001$; HFD-OS3, $p < 0.001$; HFD-SIM, $p < 0.01$); and day 3 (control, $p < 0.01$; HFD-OS1, $p < 0.01$; HFD-OS2, $p < 0.01$; HFD-OS3, $p < 0.01$; HFD-SIM, $p < 0.001$) of the acquisition trials. Whereas, during probe trial, the control and HFD-OS3 groups significantly spent more time in the platform zone (control, $p < 0.01$; HFD-OS3, $p < 0.01$) and also produced higher number of entries into the platform zone (control, $p < 0.05$; HFD-OS3, $p < 0.01$).

TABLE 1. Animal diet content

On caloric basis (in 100 g)	Cereal-based diet (1314)	Control diet with 10% energy from fat (C1090-10)	High fat diet (HFD) with 60% energy from fat (C1090-60)
Moisture	11.1%	7.9%	2.9%
Crude Ash	6.1%	4.3%	3.2%
Crude Fiber	4.5%	3.1%	4.7%
Crude Fat	5.1%	4.0%	35.0%
Crude Protein	22.5%	20.7%	21.0%
Nitrogen free extractives	50.7%	60%	33.2%
Total calories	334 kcal/100 g	351 kcal/100 g	523 kcal/100 g

TABLE 2. Daily food intake, calorie consumption and serum lipid profile

Group	Food intake (g/day)	Calorie intake (kcal/day)	Total cholesterol (mmol/L)	Triglycerides (mmol/L)	LDL cholesterol (mmol/L)
Control	3.7 ± 0.2	13.1 ± 0.9	2.3 ± 0.5	0.9 ± 0.3	0.6 ± 0.2
HFD	3.0 ± 0.4*	15.6 ± 1.9*	3.4 ± 0.6*	1.1 ± 0.4	1.3 ± 0.3*
HFD-SIM	3.2 ± 0.1*	15.8 ± 0.6*	3.4 ± 0.5*	1.0 ± 0.3	1.2 ± 0.3*
HFD-OS1	3.1 ± 0.2*	16.1 ± 1.2*	3.7 ± 0.3*	0.9 ± 0.2	1.6 ± 0.2*
HFD-OS2	3.2 ± 0.4*	16.7 ± 2.0*	3.5 ± 0.2*	1.1 ± 0.5	1.3 ± 0.3*
HFD-OS3	3.0 ± 0.2*	15.8 ± 1.0*	3.7 ± 0.5*	0.9 ± 0.2	1.4 ± 0.2*

*p < 0.05, compared to the control group. Daily food intake: gram per day food consumed; Calorie intake: Total amount of calorie (kcal) consumed per day; Serum lipid profile: Serum level of total cholesterol, triglycerides and LDL cholesterol in mmol/L

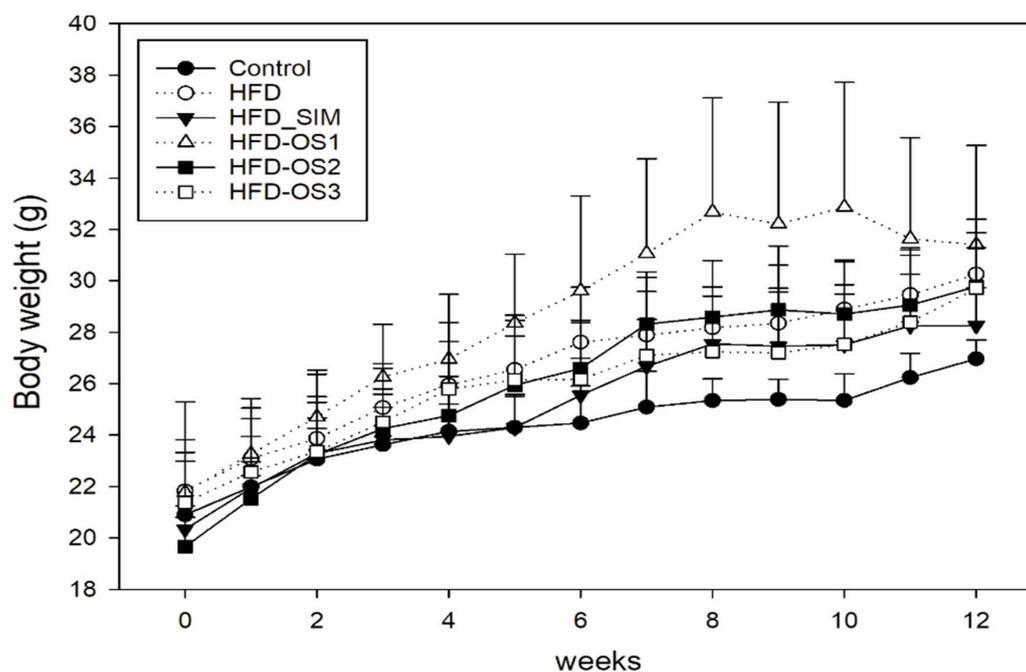


FIGURE 2. Body weight of the C57BL/6J mice during the experimental period. The data is presented as mean ± S.E.M (n=6)

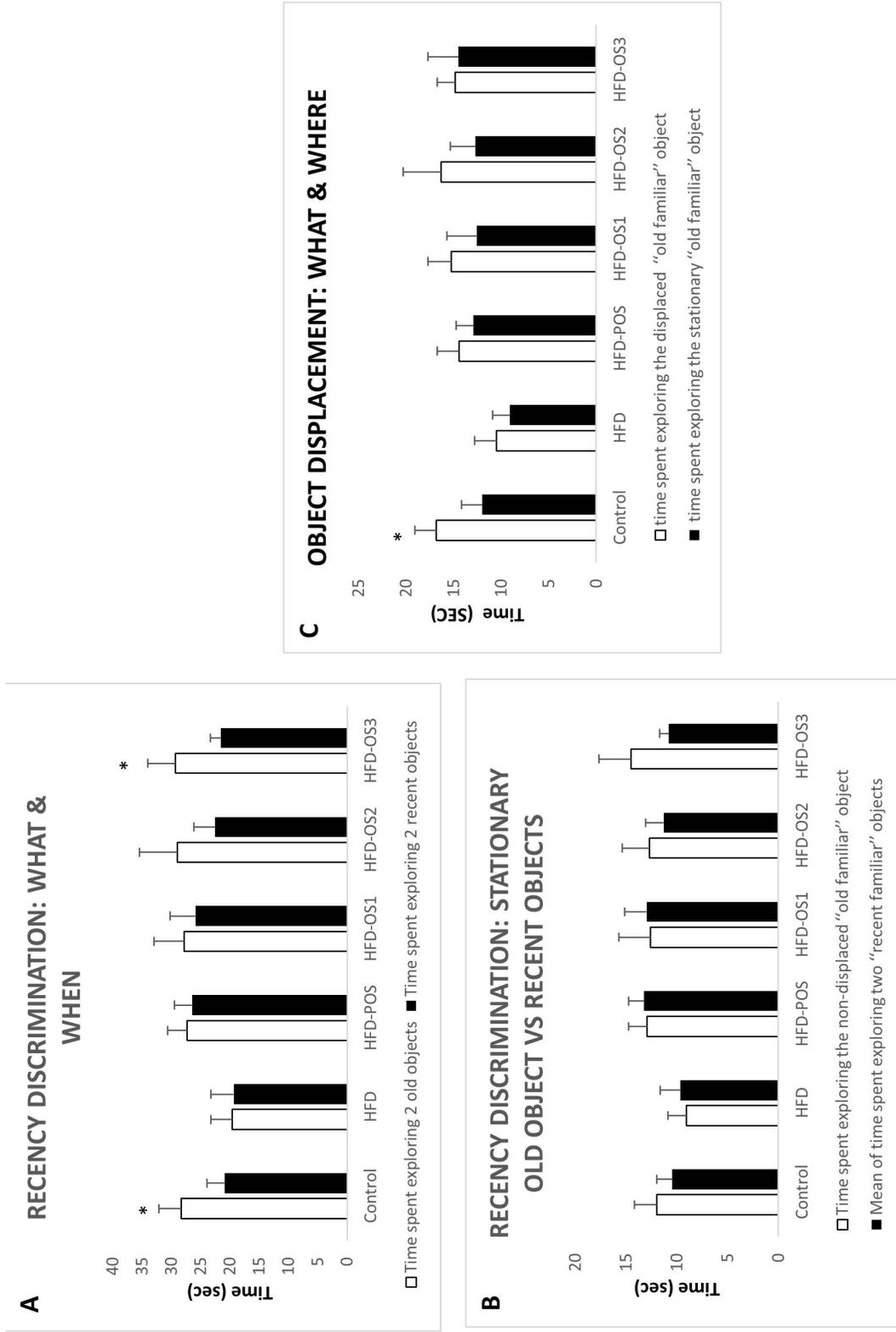
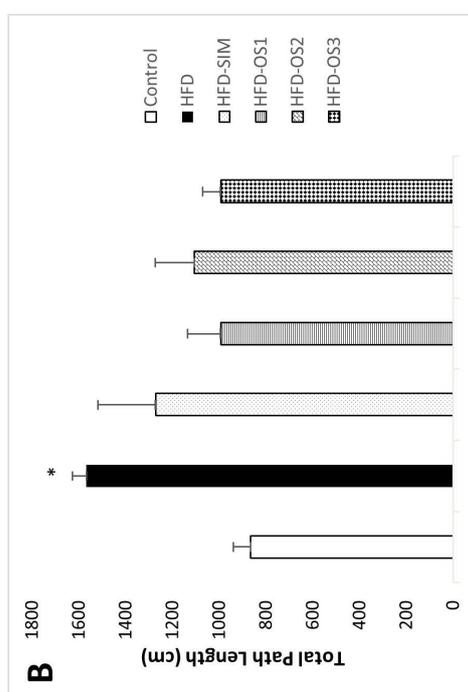
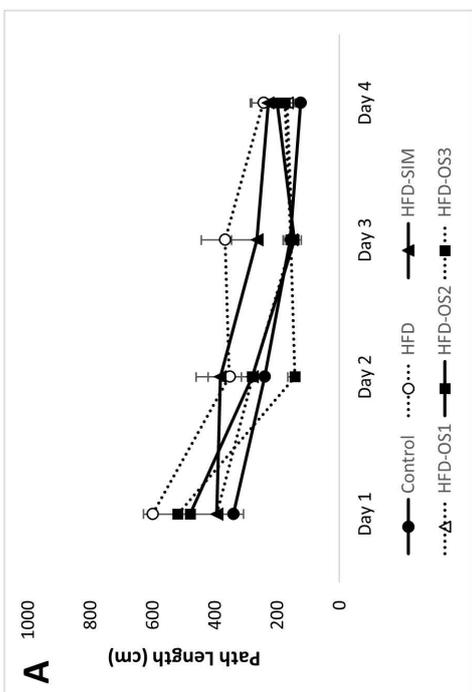
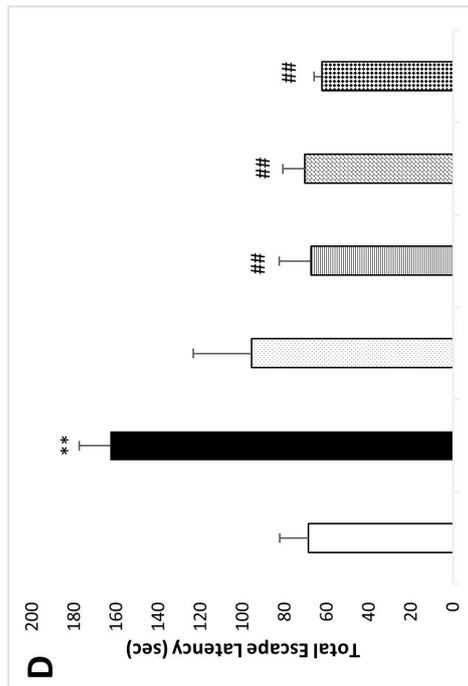
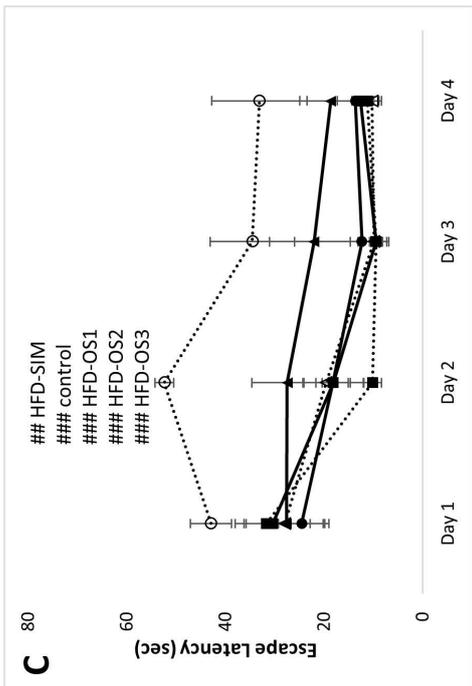


FIGURE 3. Episodic-like object memory in mice. Bar represents mean time exploring the objects. A) ‘What and when’ discrimination: Mean time spent exploring the 2 old and recent objects during the test trials. B) Recency discrimination: Mean time spent exploring the non-displaced ‘old familiar’ object and the mean of the two ‘recent familiar’ objects during the test trials. C) ‘What and where’ recognition test: Mean time spent exploring the displaced and stationary ‘old familiar’ object during the test trial. The data is presented as mean \pm S.E.M (n=6). *p < 0.05, one-tailed t-test for dependent groups



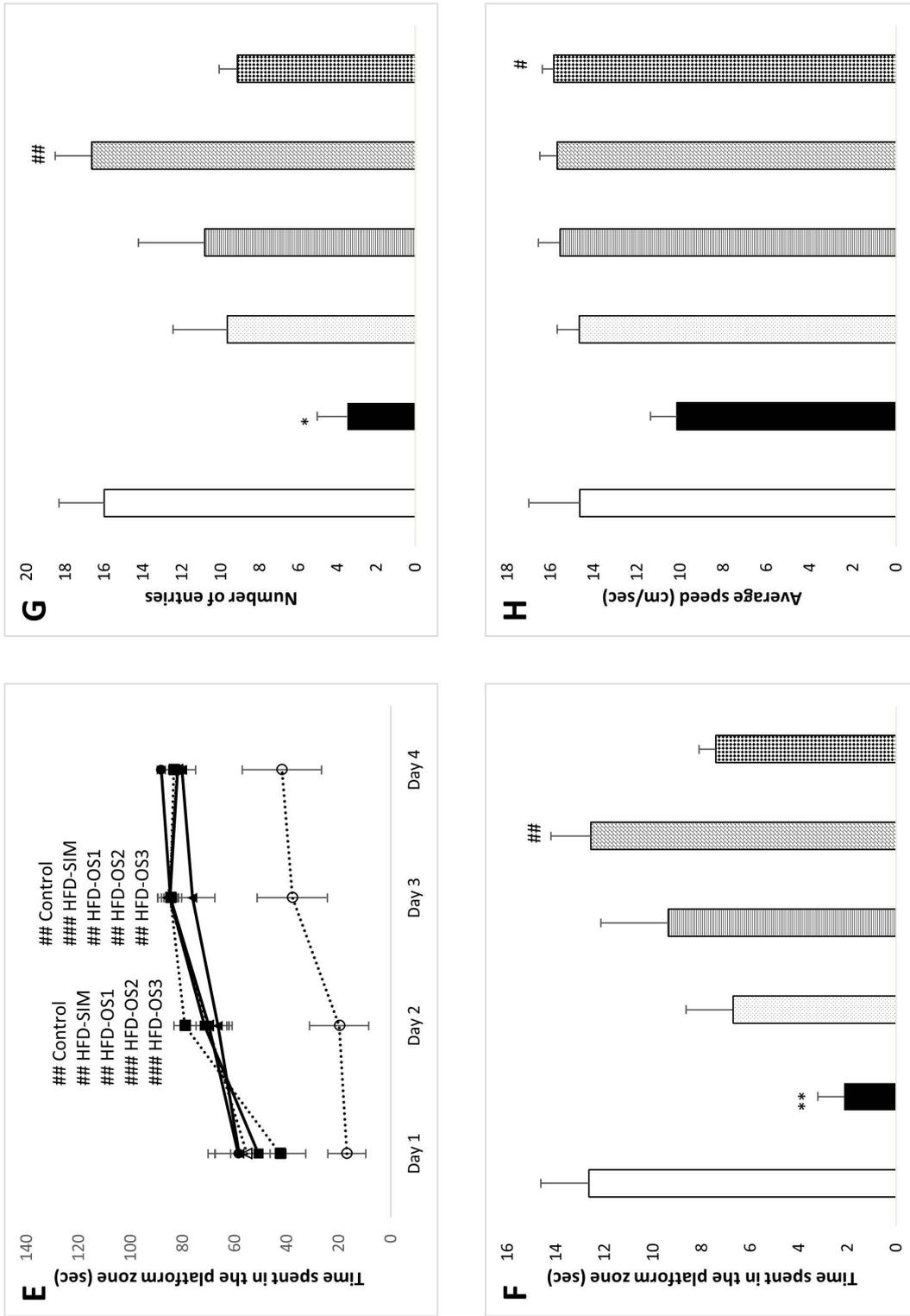


FIGURE 4. Morris water maze results. A) Path length to the platform during 4 days of acquisition trials B) Total path length to find the platform in 4 days of acquisition trials C) Escape latency: Time taken to find the platform during 4 days of acquisition trials D) Total escape latency: Total time taken to find the platform in 4 days of acquisition trials E) Time spent in the platform zone during 4 days of acquisition trials F) Time spent in the platform zone during the probe trial G) No of entries produced into the platform zone during the probe trial H) Average swimming speed during 4 days of acquisition trials. The data are presented as mean \pm S.E.M (n=6). *p < 0.05 compared to control group, **p < 0.01 compared to control group, ###p < 0.001 compared to HFD group, ##p < 0.01 compared to HFD group, #p < 0.05 compared to HFD group

DISCUSSION

In the present study, 8 weeks old male C57BL/J6 mice were fed HFD for 12 weeks with or without the supplementation of low (200 mg/kg/day), mid (400 mg/kg/day), and high (800 mg/kg/day) dosages of okra seed extract. The mice fed HFD weighed higher than the control animals, and their blood lipid markers such as LDL levels and total cholesterol were significantly elevated. The findings are similar to our previous report (Prom-In et al. 2020). Nonetheless, lipid lowering activity of OS was not noticed, as the serum lipid level was not improved compared to the HFD group, which could be due to the lower amount of polysaccharide in OS (Cahyana & Kam 2016; de Alvarenga Pinto Cotrim, Caron Mottin & Ayres 2016). Kahlon, Chapman and Smith (2007) reported that one of the mechanisms mediating the hypolipidemic effect of okra was through bile acid binding ability. Polysaccharide in okra extract traps and hinders the reabsorption of bile acid back into the bloodstream, which promotes decomposition of cholesterol into bile acid and lowers the cholesterol level. This hypothesis was in accordance with the results from a previous study, in which consumption of HFD supplemented with okra peel (main source of polysaccharide) reduced serum total cholesterol, triglyceride and LDL cholesterol (Prom-In et al. 2020). In the current study, simvastatin (20 mg/kg/day) did not affect the mice body weight and blood lipid profile profoundly, which is parallel to some past findings (Ouweneel et al. 2017; Prom-In et al. 2020; Sparrow et al. 2021), probably owing to strong compensatory increase in HMG-CoA reductase in mice (Kita, Brown & Goldstein 1980).

In mice, chronic intake of HFD is associated with impaired hippocampal-dependent spatial memory (Heyward et al. 2012; Prom-In et al. 2020). In the present study, we employed an episodic like memory test based on Dare et al. (2005b), which explores the non-verbal animals' ability to remember the 'where', 'what', and 'when' components of episodic memory, by assessing the animal's ability to recognise objects, location of objects and the temporal order memory for object presentations. As reported by Dare et al. (2005b), the control animals in our study explored the 'old familiar' objects relative to the two 'recent familiar' objects, and spatially displaced 'old familiar' objects relative to the stationary 'old familiar' objects, however, the preference ratio against chance (0.5) was only significant for recency discrimination (Figure 3). We did not find any HFD-induced significant changes

in any three components of the episodic-like memory test (there is no significant difference between the HFD and control group in any of the preference ratios). Even though the HFD-fed mice treated with the highest dose of okra seed significantly explored the 2 old objects, their preference ratio against chance was not significant. In sum, our results indicate that neither HFD nor OS had any significant effect on episodic-like memory test. Episodic memory is less commonly investigated in mice models of HFD, with a high number of studies investigating one or two components of the episodic-like memory. In line with this, two weeks of HFD reduced episodic memory concurrently with spatial memory task and contextual memory task (McLean et al. 2018). Ten weeks of high fat high fructose diet reduced discrimination index, exploration time in recognition memory task (Martinez Orozco et al. 2021). Whereas, some have reported no changes in novel object recognition even following 22 weeks of HFD intake in mice (Heyward et al. 2012).

The same groups of mice were also exposed to MWM to test their spatial learning ability during repeated acquisition trials, and reference memory when the platform is removed (Kim & Ryu 2008; Vorhees & Williams 2006). Our MWM results show that in general all the groups showed improvement in navigating through the water maze to locate the platform during the acquisition trials. We also noticed HFD-induced impairment of spatial learning (Figure 4(B), 4(C), 4(D), and 4(E)) and retention of reference memory in probe trial (Figure 4(G), and 4(H)). Our findings are similar to past studies that have reported HFD-induced cognitive impairment using MWM (Prom-In et al. 2020; Robison et al. 2020; Saiyasit et al. 2020). Simvastatin significantly improved spatial learning on day 2 (Figure 4(C)), however, in overall it doesn't have a profound impact on spatial learning and retention of reference memory. Whereas, all three doses of OS significantly improved spatial learning (Figure 4(C), 4(D), and 4(E)), and only the mid-dose of OS markedly improved the retention of reference memory (Figure 4(F), and 4(G)). We noticed no significant difference in the average speed between the control and OS-treated animals, however, HFD fed mice treated with the highest dose of OS moved significantly faster than the HFD group. Nevertheless, no significant difference was noticed following supplementation with low and mid dose of OS. Therefore, the significant effect of these dosages is not confounded by non-specific effects on locomotion.

Various parts of okra, such as the flower, peel, leaves, seed, and pod consist of bioactive compounds, including rutin, ascorbic acid, flavanol glycosides, polysaccharides, quercetin, and beta carotene exhibit an anti-oxidant, anti-inflammatory, anti-lipidemic and neuroprotective effects (Arapitsas 2008; Dubey & Mishra 2017; Ngoc et al. 2008; Tongjaroenbuangam et al. 2011). In line with this, Jasdave et al. 2017 reported that okra seed extract contains glutathione, saponins, terpenoids, polyphenols, and tannins with anti-oxidant property, which could have mediated the effects of OS we noticed in the MWM test. Existing literature correlates the pathways of neuroinflammation (Cavaliere et al. 2019; Park et al. 2010), oxidative stress-neurogenesis (Robison et al. 2020), and insulin/PI3K/Akt/Tau/Bax (Bhat & Thirumangalakudi 2013; You, Jang & Kim 2020) in HFD-induced neuronal injury. In line with this, the aqueous extract of OS alleviated depression by increasing antioxidant activities and the levels of neurotransmitters like norepinephrine, epinephrine, serotonin, and dopamine in the mice hippocampus (Vijayakumar et al. 2012). Other parts of okra were reported to reverse various hippocampal insults through pathways such as PI3K/AKT (Yan et al. 2020), neurogenesis (Tongjaroenbuangam et al. 2011), and neuroinflammation (Yan et al. 2020). At present, our results indicate that the OS's improvement of spatial memory in MWM is not due to lipid lowering effect, nor by directly influencing the mice's appetitive behaviour. We hypothesise the OS ameliorative effect could be due to its actions on neuroinflammation, insulin signaling or alteration of hippocampal neurotransmitter levels.

In the present study, HFD fed animals showed significant impairment of spatial learning and memory in MWM, however, their episodic-like memory was not significantly affected. Some researchers have reported significant deficit in object recognition but not spatial memory in HFD-fed mice (Carey, Gomes & Shukitt-Hale 2014: 11-13 months old male mice fed HFD for 5 months; Kang, Wang & Oteiza 2020: 16-18 weeks old male mice fed HFD for 13 weeks). Some have reported HFD-induced spatial memory impairment, but not Novel Object Recognition (NOR) performance (Heyward et al. 2012: 32-34 weeks old male mice fed HFD for 22 weeks). In our study, the mice were 10-12 weeks old during the cognitive assessment, thus, there's a possibility of age differences could have contributed to the mixed results in the present and past findings. It is also important to take note that, different mnemonic behavioural paradigms test different regions of the brain, for instance NOR associated with perirhinal cortex (Sharma, Rakoczy & Brown-Borg 2010), whereas, the

episodic-like memory due to its complexity is functionally associated with various regions such as the hippocampus, medial prefrontal cortex, retrosplenial cortex, and entorhinal cortex (Dere et al. 2005b). In addition to this, methodological differences in MWM between various labs, especially the duration of acquisition trials and the frequency of trials per day, and the location of platform zones during the probe trial also may have influenced the animals' learning ability and their ability to recall the reference memory.

CONCLUSION

All three doses of okra seem to improve hippocampal-related spatial learning, and only the mid dose enhanced the retrieval of reference memory in the probe trial of MWM. Despite this, none of the treatment groups performed significantly better than the HFD in the episodic-like memory test which involves both hippocampal and extra-hippocampal networks, mimicking the state of human memory. We warrant more studies in future to look into various aspects of cognitive function to have a clearer understanding on the potential cognitive enhancing or neuroprotective effect of okra seed.

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