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RESEARCH ARTICLE

Exercise and Supplementation of Black Mulberry Fruit Extract, Sunflower Seed and Pumpkin Seed Enhance Cognitive Performance among Sedentary University Students

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Abstract: ***Background*:** A sedentary lifestyle has been found to influence cognitive impairment, whereas physical activity and diet have been found to reduce the negative impacts of a sedentary lifestyle.

***Objectives*:** This study aims to examine the synergistic effects of the black mulberry, sunflower seed, and pumpkin seed supplements, including exercise on cognitive performance among sedentary university students.

***Subjects and Methods*:** Participants (n=36) were randomly assigned to receive black mulberry, sunflower seed, and pumpkin seed with and without exercise for 60 days. Then, they were required to complete cognitive task assessment for domain attention (visual reaction time and auditory reaction time), perception (fast counting and basic music) and executive (Eriksen flanker task and Stroop test) on day 0 (baseline), 30, and 60 of the experiment. Following that, blood samples were collected and analyzed for malondialdehyde serum concentration as an oxidative stress marker.

***Results*:** All participants showed significant faster reaction time in cognitive tasks for domain attention and basic music test for domain perception on day 30 and 60 compared to day 0. However, no significant changes were observed within group, on cognitive task for domain executive. In comparison between the groups, participants in group treatment showed significant faster reaction time for cognitive tasks for domain attention and for Erikson flanker task for domain executive as compared to group exercise alone, and group treatment with exercise. For domain perception (basic music test), participants in group treatment with exercise achieved faster reaction time compared to group treatment and exercise alone. Despite the reduction of malondialdehyde concentration in all groups, no significant difference was found between them.

***Conclusion*:** It was indicated from the findings that the consumption of black mulberry fruit extract, sunflower seeds, and pumpkin seeds with and without exercise independently enhanced attention, perception, and executive function among sedentary young adults.

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1. INTRODUCTION

A sedentary lifestyle is defined as a type of lifestyle where an individual has minimal or zero involvement in physical activity. It has been proven to be one of the risks for cognitive impairment [1] such as memory decline [2], delayed word recall and verbal fluency [3], resulting in neurodegenerative impact [4]. Meanwhile, physical activity has been constantly proven to enhance cognitive function [5-7]. Over 60% of the population worldwide is not engaged in moderate physical activity [8]. Based on a current report by National Health and Morbidity Survey on Adolescent Health Survey 2017, it was found that 80.2% of youngsters in Malaysia were not physically active, and 50.1% spent three hours daily in sitting activity [9]. With an exception for university students majoring in physical education, university students were observed to seldom engage in physical activities. It was hypothesized that the increasing use of smartphone and computer games were the primary factors of this situation.

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It was reported in a recent review [10] that anthocyanin-rich fruits exhibited strong antioxidant power and capability to protect the brain against cognitive deficits. This could be seen from a study where mice were fed with polyphenol-rich wild blueberry extract for 7 days. As a result, significant improvement was found in them in terms of learning and memory, with a reduction in lipid peroxidation products, brain ascorbic acid, and glutathione levels [11]. Hippocampal synaptic plasticity was also present in young rats that had been feed with blueberries for 3 weeks [12]. Meanwhile, those supplemented with pure anthocyanins for 6 weeks showed improvements in spatial memory [13].

Black mulberry (*Morus nigra* L.) is majorly distributed in Asia, South and North America, South Europe, and several parts of Africa and China. It is also known as Xiatutu, which is specified as the medicinal mulberry present in the Uygur region [14]. It possesses medicinal potential, including antioxidant [15] and anti-cancer effects [16]. The major phenolic compound groups present in black mulberry fruits are benzoic acid derivatives, cinnamic acid derivatives, flavonols, and anthocyanins [17]. The level of anthocyanin in this fruit's compound groups is represented by the dark colour [18], and these species possess higher anthocyanin compared to other types of mulberry [19]. As of recently, only one study made use of black mulberry leaf extract for cognitive impairment and oxidative stress in ageing mice [15]. Hence, it has been proved that there is no such study conducted using black mulberry fruit extract for enhancing cognitive performance, specifically in humans.

Hypothetically, a combination approach of anthocyanin-rich mulberry with another strong antioxidant, particularly vitamin E might produce a stronger effect on cognitive functioning compared to an individual approach. Several studies have shown that vitamin E accelerates learning functions, prevents memory deficit [20] and improves cognitive deficits caused by ageing [21]. In the present study, sunflower seeds and pumpkin seeds were used as the source of vitamin E due to the content of more than one vitamin E isomer [22, 23].

Due to strong potential for enhancing cognitive function, this study investigated whether a combination of exercise, black mulberry fruit extract, sunflower seeds and pumpkin seeds could produce synergistic effects on the improvement of young adults' cognitive performance.

2. MATERIALS AND METHODS

2.1. Materials

Hundred percent pure Dr Sweet™ black mulberry fruit extract was purchased from SteviaSugar Corporation (M) SDN BHD, Malaysia. The raw sunflower and pumpkin seeds were obtained from Zhejiang, China.

2.2. Subject

Thirty-six healthy female students, aged from 19 to 24 years old (mean age: 21.5 years old, SD: 1.87) from Sultan Idris Education University, Tanjung Malim, Malaysia, were selected. All of them led a sedentary lifestyle, and the selection of the students was based on the following criteria:

1) Free from social drug, alcohol, prescription medication, and herbal extract/food supplement use.
2) Free from food allergies and intolerances, and digestive problems.
3) Neither a cognitive enhancing supplement user nor excessive caffeine consumer (>6 cups of coffee/d or >450mg caffeine/day).
4) Does not have a history or presence of head injury, neurological disorder, and clinically significant cardiac, renal, hepatic, endocrine, pulmonary, gastrointestinal, or pancreatic diseases.
5) Currently not pregnant nor planning for pregnancy.

2.3. Protocol

Prior to this study procedures, these study protocols were approved by the International Islamic University of Malaysia Research Ethics Committee (IREC 2018-301) and written informed consent was obtained from all subjects. All subjects were involved in a cognitive task assessment for three consecutive days. They were then assigned into four groups (n: 8/group), each representing 1) negative control, 2) treatment (100mg/kg body weight black mulberry+50mg/kg body weight sunflower seed+50mg/kg body weight pumpkin seed), 3) exercise (30 minutes of slow run, with heart rate being monitored between 128-130bpm), and 4) treatment and exercise (100mg/kg body weight black mulberry+50mg/kg body weight sunflower seed+50mg/kg body weight pumpkin seed+30 minutes of slow run). The treatment were consumed orally twice a day for a period of 60 days. Specifically, one dose was taken after waking up in the morning, while the next dose was taken before sleeping at night. Furthermore, the exercise was done 3 times per week for 8 weeks. All subjects were required to complete the cognitive task assessment on day 0 (baseline), and on day 30 and 60 of the experiment. This assessment was conducted from 8 to 10 am when the subjects were in a fasting state. On the last day of the experiment (day 60), blood samples were collected from all subjects for oxidative stress and stress hormone marker analyses after the assessment.

2.4. Cognitive Assessment

The cognitive task battery used in this study was accessed through the website http://cognitivefun.net, which is commercially known as Cognitive Fun. Three cognitive domains were assessed in this study, namely attentional (visual reaction time and auditory reaction time), perceptual (fast counting and basic music), and executive (Eriksen flanker test and Stroop test). Following are the details of each task:

2.4.1. Visual Reaction Time

The subject was represented with a small red dot on the screen. When a large green dot appeared on the screen, subjects were required to click on it as fast as they could. Accordingly, their reaction time was recorded in milliseconds. Reaction time is an important indicator of attention and a common measure used in more complex tasks.

2.4.2. Auditory Reaction Time

In this task, auditory stimuli were used instead of visual stimuli. The subjects were required to focus on the sound. Accordingly, they were required to click on the small red dot on their screen as fast as they could when the sound was heard.

2.4.3. Fast Counting

This test involved subitising, which measured subjects' familiarity with small quantities of objects. A few black dots appeared on the screen, and subjects were required to press the number on the keyboard, which represented the amount of the appeared dots within a second.

2.4.4. Basic Music

In this test, subjects were required to be familiar with the sound of seven different piano notes. As the sound was heard, they needed to press the correct number from 1 to 7 on the keyboard to represent the correct note.

2.4.5. Eriksen Flanker Task

The flanker test was another interference task, where different inputs competed with the target, which slowed down the response speed. Subjects were required to identify the direction of the center arrow and indicate so by pressing on the respective arrow on the keyboard as fast as possible.

2.4.6. Stroop Test

This test involved the dissonance between the colour presented on the screen and the mismatched name to interfere with the colour naming. Subjects were required to press the first letter of the respective name of colour which appeared on their screen. For example, the letter R was pressed for red, the letter Y for yellow, and many other instances.

2.5. Oxidative Stress Marker

To examine the serum, blood samples collected from each subject were centrifuged at 3500rpm for 15 minutes. This serum was used to determine Malondialdehyde (MDA) concentration through the ELISA kit (Finetets, China), according to the manufacturer's instruction.

2.6. Statistical Analysis

All data were analysed using a one-way analysis of variance (ANOVA). The data were expressed as mean±standard deviation. Differences between the groups were evaluated by LSD post hoc test and considered significant at $p<0.05$. All statistical analyses were performed using SPSS 25 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1. Effects on Cognitive Domain Performance

Subjects' cognitive performances in terms of attention, perception, and executive domains are presented in Figs. (**1a-1c**), respectively.

Attentional Domain -Based on the visual reaction time graph (Fig. **1a**), the time taken for the subjects in group treatment and group exercise to react towards the stimulus significantly reduced on day 30 (Trt, 0.37±0.09s, p=0.021, 42%; Ex, 0.47±0.15s, p=0.029, 27%) and day 60 (Trt, 0.33±0.07s, p=0.009, 49%; Ex, 0.43±0.1s, p=0.007, 34%) as compared to day 0 (Trt, 0.64±0.38s; Ex, 0.64±0.21s). Based on the group comparison, the group treatment showed faster reaction time by 23% and 33% on day 60 compared to group exercise (p=0.033) and treatment+exercise (p=0.002) respectively. Based on the auditory reaction time graph (Fig. **1a**), significant effects were observed among the subjects from all groups on day 30 (Trt, 0.26±0.07s, p=0.041, 46%; Ex, 0.42±0.09s, p=0.001, 29%; Trt+Ex, 0.38±0.11s, p=0.000, 65%) and day 60 (Trt, 0.23±0.06s, p=0.020, 53%; Ex, 0.29±0.05s, p=0.000, 50%; Trt+Ex, 0.43±0.19s, p=0.000, 60%), compared to day 0 (Trt, 0.48±0.36s; Ex, 0.59±0.11s; Trt+Ex, 1.08±0.35s). Moreover, faster reaction time could be seen in the group treatment compared to group exercise (day 30, p=0.002, 37%), and group treatment+exercise (day 30, p=0.016, 32%; day 60, p=0.002, 47%). On day 60, the group exercise also displayed a significant effect compared to group treatment+exercise (p=0.029, 32%).

Perceptual- Based on the fast counting graph in Fig. (**1b**), compared to day 0 (1.13±0.15s), significant effects were observed only in subjects supplemented with treatment on day 60 (0.9±0.1s, p=0.001, 20%), and no significant difference was shown in comparison between the groups. Meanwhile, the basic music graph in Fig. (**1b**) shows significant effects on all groups on day 30 (Trt, 1.8±0.34s, p=0.043, 14%; Ex, 1.76±0.8s, p=0.012, 29%; Trt+Ex, 0.98±0.21s, p=0.001, 50%) and day 60 (Trt, 1.55±0.31s, p=0.001, 26%; Ex, 1.23±0.59s, p=0.000, 55%; Trt+Ex, 0.96±0.1s, p=0.001, 51%) compared to day 0 (Trt, 2.1±0.25s; Ex, 2.71±0.68s; Trt+Ex, 1.97±0.86s). Based on the group comparison, group treatment+exercise showed faster reaction time compared to the group treatment (day 30, p=0.003, 45%; day 60, p=0.004, 38%), and group exercise (day 30, p=0.006, 44%).

Executive- Based on the Eriksen flanker task graph in Fig. (**1c**), no significant effect was found within the group. However, the subjects who were provided with treatment (0.51±0.05s) showed a 21% faster reaction compared to group treatment+exercise (0.64±0.13s, p=0.024) on day 60. Group treatment also showed faster reaction time by 28% on day 30 (0.57±0.11s, p=0.024) and 21% on day 60 (0.51±0.05s, p=0.023) compared to the group exercise (day 30, 0.79±0.29s; day 60, 0.64±0.15s). Nevertheless, no significant difference was observed for both within and between groups in the Stroop test.

3.2. Effects on Oxidative Stress Marker

No significant changes in serum MDA concentration were seen among all groups (Trt, 16.73±0.26; Ex, 16.92±0.9; Trt+Ex, 17.18±1.04) even though the subjects supplemented with treatment showed 4% reduction, while the exercise group showed 3% reduction as compared to control group (17.49±1.81) (Fig. **2**).

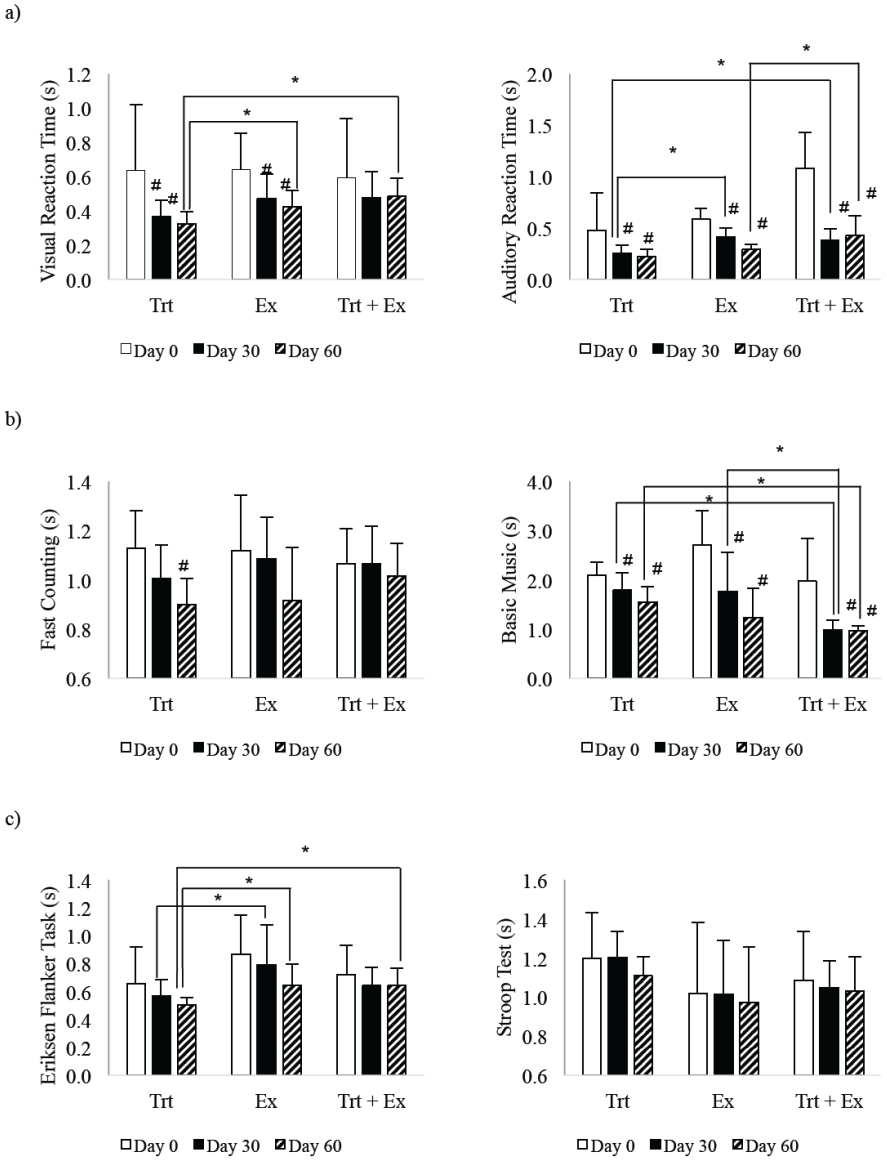

Fig. (1). Effects of Trt, Ex, and Trt+Ex on cognitive assessment (**a**) domain attentional: visual reaction time and auditory reaction time, (**b**) domain perceptual: fast counting and basic music, (**c**) domain executive: Eriksen Flanker task and Stroop test. Lower score denote greater domain cognitive performance. Data are expressed as the mean±SD. #p<0.05 (significant difference within group, compared with day 0), *p<0.05 (significant difference between group, comparison between each particular day). Trt, Treatment (black mulberry, sunflower seed and pumpkin seed); Ex, Exercise (30 minutes of slow run); Trt+Ex, Treatment with exercise (black mulberry, sunflower seed and pumpkin seed with 30 minutes of slow run).

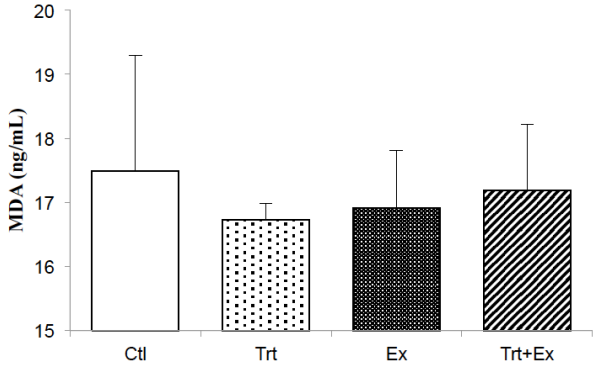

Fig. (2). Effects of Trt, Ex, and Trt+Ex on serum malondialdehyde concentration. Shorter bar represent better therapeutic effect on oxidative stress marker. Ctl, Control (no intervention); Trt, Treatment (black mulberry, sunflower seed and pumpkin seed); Ex, Exercise (30 minutes of slow run); Trt+Ex, Treatment with exercise (black mulberry, sunflower seed and pumpkin seed with 30 minutes of slow run).

4. DISCUSSION

A sedentary lifestyle is linked to various negative health conditions, including cognitive decline. Therefore, this study aims to prove that exercise, along with the nutrition from antioxidant sources, namely black mulberry, sunflower seed and pumpkin seed could enhance cognitive performance, specifically in terms of domain attention, perception, and executive, by functioning synergistically with one another.

In the case of the domains, attention refers to individual's capacity to maintain focus on a specific item or a task for an extended period of time [24], making it essential in the learning process. Meanwhile, perception is one's ability to capture, process, and actively make sense of the information received by the senses. Perception is a cognitive process that enables the interpretation of one's surroundings through the stimuli received by sensory organs. Following that, executive function is responsible for more complex cognitive functions and decision making, which are essential for one's reading ability [25] and academic achievement [26].

In the present study, it was proven that cognitive performance for attention, perception, and executive domains improved after 30 days and 60 days of black mulberry fruit, sunflower seed, and pumpkin seed consumption. This improvement occurred with and without exercise combination. Notably, with respect to domains of attention and executive, the subjects who were only provided with supplements exhibited faster reaction time compared to those who performed exercises. In the case of domain perception, the group treatment with exercise showed faster reaction time compared group treatment and exercise alone. Overall, it was found that treatment and exercise each could improve cognitive performance without synergistic interaction with each other.

The aforementioned results are in agreement with the findings reported by Turgut *et al*., [15], who investigated the effects of black mulberry treatment on cognitive impairment among D-galactose-induced ageing mice. It was found that the mice's learning dysfunctions significantly improved after 60 days of treatment with black mulberry. Watson and colleagues found that acute administration of anthocyanin-rich blackcurrant juice led to higher alertness levels and reduced fatigue [27]. It also improved sustained attention and psychomotor speed among healthy young adults [28]. Considering the influence of exercise on cognition improvement, it was found that subjects who were regularly performing aerobic exercise [7] and sprinter-based exercise [5] experienced an enhanced speed of executing tasks, with the assistance of response time in the Stroop test.

Among the major causes for the decline of cognitive function are the accumulative effects of the oxidative damage caused on cellular components in the brain [29]. Furthermore, the increase in reactive oxygen species (ROS) was reported among subjects who led a sedentary lifestyle [30]. In this study, the concentration of malondialdehyde (MDA) as an oxidative marker was reduced in the group who consumed black mulberry fruit extract, sunflower seeds, and pumpkin seeds, regardless of them performing an exercise or not. However, no significant difference was observed when compared with the control group. This finding was in agreement with the previous study, which investigated serum, brain, and the liver tissue of D-galactose-induced mice. In this case, it was found that black mulberry extract significantly reduced MDA level and increased antioxidant enzymes, namely superoxide dismutase and glutathione peroxidase after eight weeks of treatment [15].

CONCLUSION

Overall, it was indicated from this study's findings that exercise and the consumption of black mulberry fruit extract, sunflower seeds, and pumpkin seeds can be useful in improving the attention, perception, and executive functions among sedentary young adults, regardless of the combined approach or vise versa. These interventions might be useful, especially for school and university students, to enhance their cognitive functions, thus helping them to achieve success in academic life. It is recommended that future studies determine the molecular measures, such as neuroplasticity markers, endocrine system, and brain imaging.

LIST OF ABBREVIATIONS

MDA = Malondialdehyde

ROS = Reactive Oxygen Species

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocols and consent form were approved by the International Islamic University of Malaysia Research Ethics Committee (IREC 2018-301).

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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