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# Sheep Placental Extract Improves Memory and Exercise Performance in Senescence-Accelerated Mice

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**Key words**
**Sheep placenta, Exercise, Memory, Ageing, SAMP8**



## ABSTRACT

Traditional and folk medicine has used the placenta to treat a wide range of ailments. Fatigue is one of the frequent physiological reactions resulting from hard physical and mental work or severe life stress. The present study aimed to evaluate the effect of sheep placental extract (SPE) on memory and exercise performance in aged mice. Senescence-accelerated mouse prone-8 (SAMP8) was used as an ageing model and mice were fed with 3 (low, medium, and high) doses of SPE. Passive and active avoidance tests were performed to assess the memory function, treadmill exercise, forelimb grip strength test, liver glycogen and serum lactate were measured to assess the exercise performance. The memory of the aged mice was improved after SPE administration. Further, SPE treatment increased exercise performance by increasing forelimb grip strength and liver glycogen levels and decreasing serum lactate levels. To conclude, feeding sheep placental extract improves learning, memory, and exercise performance in ageing mice.

## INTRODUCTION

The placenta is a vascular organ with a known lifespan that only develops during pregnancy. It affixes to the uterine wall and the umbilical cord, which bonds the mother with the fetus, facilitating the fetus's growth and development (Kumar *et al*., 2020). They are crucial for the fetus's survival thoughout pregnancy because they help transport gases, nutrients, and waste out of the body (Aplin *et al*., 2020; Rosenfeld, 2021). It has been demonstrated that administering placental extracts can enhance overall health and well-being. Iron from the placenta restores the body's supply of iron, eradicating weariness and improving energy (Mumtaz *et al*., 2022). The treatment of chonic fatigue syndrome using the human placenta is widespread in traditional Chinese medicine (Park *et al*., 2016). On parental placentophagy, 189 females were subjected to a survey. After consuming the placenta, 26% of the mothers reported having more energy and feeling less tired, 40% reported having better moods, and 15% reported having better lactation (Selander *et al*., 2013).

According to some findings, animal placental extracts performed similar activities to the human placenta. Porcine placental extract demonstrated strong immunomodulatory

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effects both *in vitro* and *in vivo*, according to Park *et al.* (2011). The proliferative response to lectins and lymphocyte activation might also be inhibited by sheep placental extract (Cotor *et al.*, 2011). In order to substitute the human placenta, domesticated animal placenta from sheep, pigs, and cows can be used. Traditional Chinese medicine has utilized sheep placenta for its pharmacological effects, and recent investigations have shown that it is an animal resource that is abundant in biological and pharmaceutical components (Young and Benyshek, 2010; Lo *et al.*, 2018; Shen *et al.*, 2022). After ultrafine grinding and freezing centrifugation, the water-soluble immuno-active peptides are often recovered as pharmaceutical components (Cotor *et al.*, 2011), and the leftover precipitate is subsequently disposed of as industrial waste. In practical terms, the precipitate, a byproduct of sheep placenta, was a rich source of proteins. These proteins have the potential to be important resources. However, antioxidant peptides derived from sheep placenta byproduct protein are not well understood (Teng *et al.*, 2011; Ming *et al.*, 2017).

Age-related learning and memory deficiencies are serious issues, and animal models with these deficits may be helpful in treating aging people in gerontological research. An animal model of accelerated senescence was developed using the senescence-accelerated mouse (SAM) strains (Takeda, 1999). The senescence-accelerated resistant mouse SAMR (SAMR1, R2 and R4) and the senescence-accelerated prone mouse - SAMP (SAMP1, P2, P3, P6, P8, P9 and P10) make up the SAM strains (Takeda *et al.*, 1997). Advanced senescence symptoms such as decreased activity, hair loss, lack of glossiness, skin coarseness, peri ophthalmic lesions, increased lord kyphosis of the spine, and a shorter life span is documented in SAMP mice. One strain of the SAM model, SAMP8, displays memory and learning deficiencies as people age (Nomura and Okuma, 1999; Ohta *et al.*, 2002; Zhou *et al.*, 2018).

One of the common physiological responses to demanding physical and mental activity or high levels of stress is fatigue (Burnley and Jones, 2018). An epidemiological study indicated that tiredness is prevalent in highly educated middle-aged persons, the majority of whom are women (Bensing *et al.*, 1999). As a result, fatigue has emerged as a prevalent health issue. It is necessary to take medicine or get psychiatric treatment to treat exhaustion since, if it is not treated for a long time, it might turn into chonic fatigue syndrome and negatively impact both job and personal life, possibly even resulting in mortality (McManimen *et al.*, 2016). Medications used to treat fatigue include biological products, herbal remedies, and chemical medications. Chemical drugs and biological products can increase exercise capacity and postpone the onset of tiredness; however, because the majority of these items are central nervous system stimulants that can lead to addiction and dependency, their therapeutic uses are restricted.

Integrative biological medicine is crucial for managing and restoring health and fitness. Placental extracts, which still retain active components, are crucial for rejuvenation, revitalization, and the restoration of youth and vitality by delaying the ageing processes. It is regarded as a powerful therapeutic agent with effective regeneration abilities during ageing. The present study aimed to evaluate the effect of sheep placental extract on memory and exercise performance in aged mice.

## MATERIALS AND METHODS

*Animals*

Male and female SAMP-8 mice were purchased from BioLASCO, Taipei, Taiwan. Thee same-sex animals were caged together. Animals are kept in 30(W) × 20(D) × 10(H) $cm^3$ transparent plastic cage, the temperature of the animal room is maintained at 22 ± 2° C, the relative humidity is 65 ± 5%, and in a dust-free automatic control room. Control the light cycle with an automatic timer, 07:00~19:00 belong to the light period (light period), 19:00~07:00 belong to the dark period (dark period). Feed and water were taken *ad libitum*.

*Sheep placenta extract preparation and dosage*

The experimental sample in this experiment is the dried sheep placenta powder provided by Zhenyuebo International Co., Ltd., which is the raw material of the placenta of the Ujimqin sheep produced in Inner Mongolia. It is manufactured by East Ujimqin Banner Yueyi Biotechnology Co., Ltd.

The dose conversion is based on the experimental evaluation method of the Ministry of Health and Welfare to first convert the recommended daily intake of human body per kilogram of body weight, and then expand the recommended human dose by 12.3 times to obtain the dose for mice, and use the medium dose as the conversion basis (Zhong *et al.*, 2019).

*Low dose group (0.5 time)*

The recommended intake for adults is 600mg/ 60 kg BW/ day × 1/2 = 5mg/ kg BW/ day. The intake for mice was 5 mg/kg BW/day x 12.3 = 61.5 mg/kg BW/day Then the daily intake of mice was estimated based on the proportion of their body weight.

*Medium dose group (1 time)*

The recommended intake for adults is 600 mg/ 60 kg BW/ day × 1 = 10 mg/ kg BW/ day. The intake for mice

was: 10 mg/kg BW/day × 12.3 = 123 mg/kg BW/day. The daily intake of mice was estimated based on the proportion of their body weight.

*High dose group (1.5 time)*

The recommended intake for adults is 600 mg/60 kg BW/day × 1.5 = 15 mg/kg BW/day. The intake for mice was 15 mg/kg BW/day × 12.3 = 184.5 mg/kg BW/day. Then the daily intake of mice was estimated based on the proportion of their body weight.

*Experimental animal grouping*

In this experiment, 3 months old male and female SAMP8 mice were used as experimental animals, and they were divided into control group and experimental group (low, medium, and high doses), 10 mice in each group. Animals were randomly divided into 4 groups (n=10), Group A - control group, Group B - low dose SPE (61.5 mg/kg BW/day), Group C - medium dose SPE (123 mg/kg BW/day), and Group D - high dose SPE (184.5 mg/kg BW/day). SPE was administered orally. Animal-experiment procedures in the present study were approved by the Institutional Animal Care and Use Committee (IACUC) of Providence University (Approval No: 20201218 A008).

*Learning and memory ability test*

*Single passive avoidance test*

For a single passive avoidance test, a 35 (W) × 17 (D) × 20 (H) $cm^3$ aluminium box (shuttle cage, Coulbourn instruments Model E10-15) was used, which was divided into a bright room and a dark room, with a 7.5 (W) × 6.5 (D) $cm^2$ of small gates are separated and communicated (guillotine door, Coulbourn instruments Model E10-15GD). The bottom of the box is provided with metal rods arranged in parallel at an interval of 1 cm and connected to a current device. During the test, the mice were first placed in the bright room, and after 10 sec of environmental adaptation, the small gate between the two rooms was opened to allow them to explore freely. Since the mice have nocturnal behaviors that tend to darken, once the mice entered the dark room, the small gate quickly opened. Close it, and after 5 sec, give a shock of 0.5 microamps for 0.5 sec, three consecutive times with an interval of 5 sec each time, that is, the learning training is completed. After the training, their memory ability was measured for 24 h and 48 h respectively. The same method was used during the test, but no electric shock was given at this time, and the time spent in the bright room was recorded. The maximum duration of each test is 180 sec. The longer the time spent in the bright room, the better the memory ability of the mice.

*Active shuttle avoidance test*

The active avoidance test uses a 35 (W) × 17 (D) × 20 (H) $cm^3$ aluminum box (shuttle cage, Coulbourn instruments Model E10-15), which is divided into two compartments with a 7.5 (W) × 7.5 (W) × Small gates of 6.5 (D) $cm^2$ are spaced apart and can communicate with each other. The bottom of the box is provided with metal rods arranged in parallel at an interval of 1 cm and is connected to a current device. The experimental process is completely controlled by computer programming to control time, sound, light and electric shock. During the test, put the mouse aside, and after 10 sec of adaptation (intertrial interval), a 10-second light and sound stimulus (conditioned stimulus; CS) will appear on the computer. If the mouse is under the CS system, it still stays in If there was no response from the same side, the computer then gave a 0.3 mA (0.3 mA) electric shock (unconditioned stimulus; UCS) for 5 sec; if the mouse entered the other side under the CS system, no electric shock was given. The computer will automatically show the status of CS and UCS according to the reaction of the mice. Each mouse receives 5 times of CS and UCS tests at one time, and then the mice are put back into the rat cage. The UCS procedure was performed 4 times for 3 consecutive days. This test explores the number of successful avoidance responses under the CS system and determines the effect of feeding each group of diets on the learning and memory ability of mice.

*Exercise performance*

All experimental mice underwent treadmill exercise, and exercise testing was performed at weeks 0, 4, 8, and 13. The exercise test method is to set the starting speed of the mice to run at 15 m/min for 20 min, rest for 90 sec, then run at 20 m/min for 20 min, rest for 90 sec, and finally run at 25 m/min. Run for 20 min. In the exercise test, the tail end of a treadmill (model T306, Diagnostic and Research Instruments Co., Taoyuan, Taiwan) was equipped with a metal ring and a conductor, and an electric shock (the current intensity was 0.6 mA for a time <2 s) was used as an excitation for mice to run behavior and simultaneously record the number of times, the treadmill incline is 0 degrees. Retention frequencies are when mice touch the electric shock area behind the treadmill due to fatigue during exercise testing, electric shocks will be administered to stimulate running and the number of times will be recorded. The number of shocks each mouse received at different speeds was recorded during exercise testing to assess motor performance.

*Forelimb grip strength test*

Using a mouse grip strength meter (Ugo Basile Grip

Strength Meter, Cat. No. 47200), it was performed 30 min after feeding in the 9th week. Using an animal forelimb grasping force measuring instrument, the mice were placed on the experimental table with a grasping rod equipped with a force sensor in front of the head, and the height of the grasping rod was adjustable. Grab 1/3 of the base of the mouse's tail, make the mouse grab the measuring device, pull it in parallel and in the opposite direction, so that the mouse's body and the sensor are level and pull the mouse's tailback until it loses grip. Repeat 3 times and take the maximum value. When dragging an animal's tail, the animal instinctively grabs the front grab bar in order to prevent unintended backward movement until the operator pulls more than its maximum grip. When the animal releases the grab bar, the instrument automatically records the maximum force.

*Statistical analysis*

The data obtained in this study were analyzed by SPSS statistical software package. The experimental data were analyzed by one-way analysis of variance (one-way ANOVA) to test the differences between multiple groups, and Duncan's multiple range test was used to compare the differences between groups. A significant difference was indicated when $p<0.05$.

## RESULTS

*Effect of SPE on passive avoidance in mice*

To access the effect of SPE on the memory of aged mice, a passive avoidance test was performed on both male and female mice (Fig. 1). On day 1 the animals were trained, and no significant changes were observed. However, in 24 h and 48 h, female mice fed with low, medium, and high doses of SPE significantly spent more time in the bright room compared to the control group. In male SAMP8 mice, after 24 h and 48 h, low dose SPE had no significant effect, but medium and high SPE doses fed mice stayed significantly more time in the bright room.

*Effect of SPE on active avoidance in mice*

Learning and memory in animals can be studied using an active avoidance test. On the first day of the experiment, there is no significant difference between the experimental groups of both sexes. On the 2nd day, medium and high SPE treatment in SAMP8 animals showed significantly increased escape response when compared to the control group. On the 3rd day, all the SPE-fed mice showed higher escape responses compared to the control group (Fig. 2).

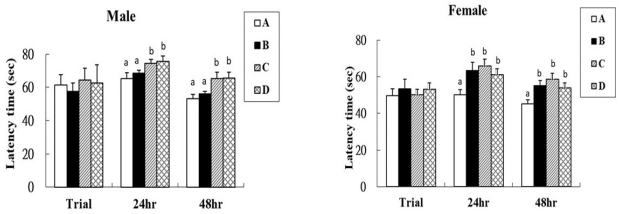


Fig. 1. Effect of SPE on passive avoidance test in SAMP8 mice. (A) Male (B) Female. Data were expressed as the mean ± SEM and analyzed by one-way ANOVA. Groups with different letters indicate significant differences among each group ($p<0.05$).

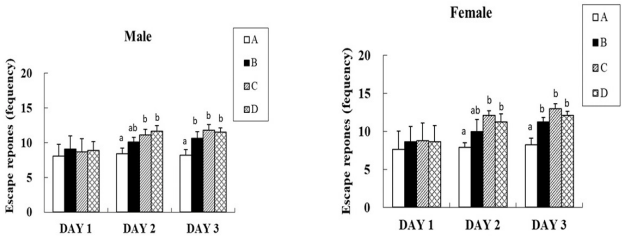


Fig. 2. Effect of SPE on active avoidance test in SAMP8 mice. (A) Male (B) Female. Data were expressed as the mean ± SEM and analyzed by one-way ANOVA. Groups with different letters indicate significant differences among each group ($p<0.05$).

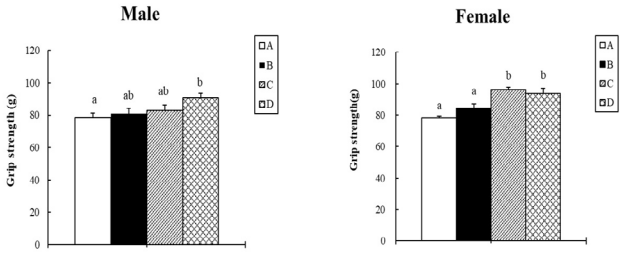


Fig. 3. SPE increases the forelimb grip strength in ageing mice. (A) Male (B) Female. Data were expressed as the mean ± SEM and analyzed by one-way ANOVA. Groups with different letters indicate significant differences among each group ($p<0.05$).

*SPE increases the forelimb grip strength in ageing mice*

The forelimb grip strength test was performed to assess the muscle strength and neuromuscular coordination of the animals. In the control group, forelimb grip strength was found to be 78.3 and 77.8 in male and female SAMP8 mice, respectively. Administration of aged mice with medium and high SPE doses significantly improves the forelimb grip strength when compared to the control, whereas low SPE dose have no significant effect in male and female mice (Fig. 3).

*SPE increases exercise performance in senescence-accelerated mice*

To evaluate the effect of SPE on exercise performance, SAMP8 mice were subjected to treadmill exercise at 3 different running speeds (15 m/min for 20 min, 20 m/min for 20 min and 25 m/min for 20 min) in week 0, 4, 8 and 13. Both male and female aged mice display similar results in treadmill exercise (Figs. 4 and 5). The results showed that at the thee running speeds, the retention frequencies of mice in each group increased with the increase in exercise speed and time in weeks 0 and 4. Since the experimental animals are in the stage of learning and adaptation there is no significant difference in the number of electric shocks in the running test between each group. During week 8 and 13, at a speed of 20m/min and 25m/min, the number of electric shocks in the high-dose sheep placenta test group was significantly lower than that in the control group ($p<0.05$); whereas low and medium doses have no significant effect.

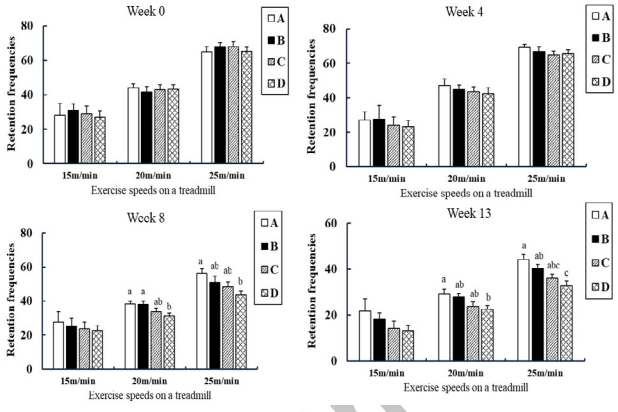


Fig. 4. SPE increases exercise performance in male senescence-accelerated mice. Data were expressed as the mean ± SEM and analyzed by one-way ANOVA. Groups with different letters indicate significant differences among each group ($p<0.05$).

*Effect of SPE on liver glycogen and serum lactate in mice*

Exercise performance needs higher glycogen in hepatic tissues. In male SAMP8 mice, medium and higher SPE doses significantly improved liver glycogen levels, and low SPE had no significant effect. In female SAMP8 mice, higher SPE significantly increased the liver glycogen levels, but low and medium SPE doses has no significant impact. Lactate levels in the serum of aged mice were lowered considerably by medium and higher SPE doses in both male and female mice when compared to their respective control groups, whereas low SPE had no significant effect in both sexes (Fig. 6).

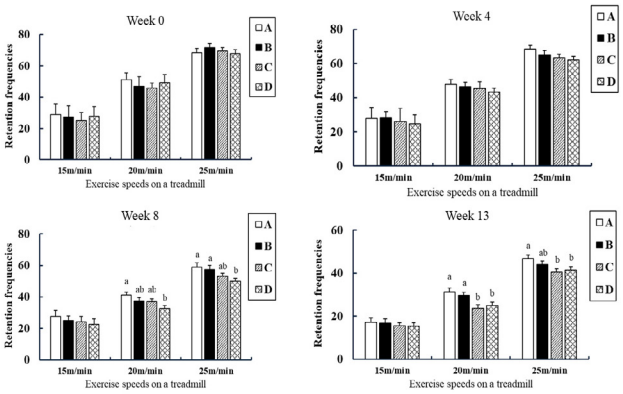


Fig. 5. SPE increases exercise performance in female senescence-accelerated mice. Data were expressed as the mean ± SEM and analyzed by one-way ANOVA. Groups with different letters indicate significant differences among each group ($p<0.05$).

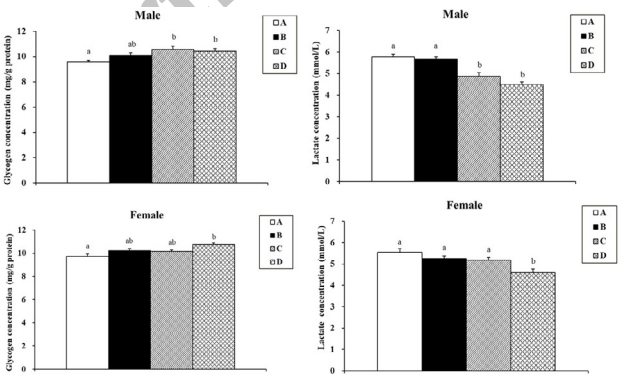


Fig. 6. Effect of SPE on liver glycogen and serum lactate in SAMP8 mice. (A) Male (B) Female. Data were expressed as the mean ± SEM and analyzed by one-way ANOVA. Groups with different letters indicate significant differences among each group ($p<0.05$).

## DISCUSSION

Placenta extract has been shown in several experiments to prevent ageing, tiredness, and a variety of other diseases (Rozanova *et al*., 2012). Various biological systems have been suggested to benefit from the placenta's potential antioxidant and immune-modulating effects (Park *et al*., 2016). Additionally, peptides present in the placental extract contain antioxidant components that can reduce ROS production, prevent exhaustion, protect mitochondrial, and prevent oxidative stress (Lee *et al*., 2011). The main amino acids in SPE were glutamic acid, aspartic acid, lysine, and leucine. Eight essential amino acids made up 31.20 g/100 g of SPE or 39.48 percentage of total amino acids. Additionally, several cytokines

and growth factors present in placenta extract were also discovered to be crucial for their pharmacological activities (Liu *et al*., 2018). It is plausible that placental extract might have a significant impact on memory and learning. Sheep placenta provides a number of medicinal advantages, such as anti-ageing, antioxidant, and anti-fatigue (Pan *et al*., 2017). The antifatigue effects of SPE and the effective mechanism have not been reported. Therefore, this study examines the effect of SPE on memory and exercise performance in aged mice.

The mice used in this investigation are SAMP8 (SAMP8) strains, which have a short lifetime and a number of ageing-related traits, including amyloid deposition, cortical atrophy, lipofuscin, brainstem reticular spongiform degeneration, and loss of brain neuronal cells (Takeda, 2009). The detrimental effects of aging include cognitive deficiencies, including learning difficulties and delayed memory. In general, memory is seen to be a process with numerous phases, including acquisition, consolidation, and retrieval. Memory is tested by animal behaviour changes sometime after learning (Abel and Lattal, 2001). Rats and mice are often tested for learning and memory using the passive and active avoidance paradigm (Cimadevilla *et al*., 2000; Cain, 2019). In the passive avoidance test, the acquisition latency (initial latency) to enter the compartment on the first day is a measure of visual function and motor activity, and the retention latency (step-though latency) to enter the compartment on the second day is a measure of memory of the intense experience. In both male and female SAMP8 mice, SPE showed an impact that improved learning memory during the passive avoidance test. In comparison to the control group, the SPE treatment considerably reduced the retention trial's latency time. The escape reaction in the active avoidance test was boosted by SPE treatment. Our research supports a prior study that found saffron improves memory and learning due to its antioxidant properties (Papandreou *et al*., 2011).

Animal model fatigue was evaluated using endurance swimming time, forelimb grip strength, changes in biochemical marker levels, and histological examination of muscle and other tissues (Tung *et al*., 2019; Chen *et al*., 2021). Forelimb-grip, rotary rod, and treadmill running tests have all been extensively employed in animal models to assess the anti-fatigue effects of medications or natural compounds (Fiuza-Luces *et al*., 2019; Chaix *et al*., 2021). The main expression of a drug's or natural compound's anti-fatigue action is an increase in exercise endurance time. In the present study, SPE treatment significantly improved forelimb-grip strength compared to the control. Forelimb-grip strength is significantly higher with a medium and higher SPE dosage compared to a lower SPE dose. This shows that the potential advantages of SPE administration have a positive impact on improving grip strength without training. Additionally, SPE markedly decreased the retention frequencies in weeks 8 and 13 compared to the corresponding controls. It has been demonstrated that administering phytochemicals such as resveratrol, capsaicin, and curcumin improves forelimb-grip strength and endurance treadmill running time in animal models without training (Wu *et al*., 2013; Huang *et al*., 2015; Hsu *et al*., 2016). Forelimb-grip strength and running duration were both improved by treating with a Chinese herbal extract from *Glossogyne tenuifolia* (Chen *et al*., 2022).

When the body does not have enough glucose available, it might quickly break down for the benefit of the body. It helps to increase muscular endurance, keep blood sugar levels stable, and make exercise last longer. As a result, the reference standard for the execution of exercise endurance is the level of hepatic glucose (Van Praag *et al*., 2014). Fatigue may set in when the material progressively disappears, which will lower athletic performance. During the activity, glucose can be utilized as a source of energy. There are two types of mechanisms for turning glycolysis into energy: aerobic and anaerobic. Rapid production of lactic acid can readily result from high-intensity exercise and increases the rate of anaerobic glycolysis. Athletic performance is also influenced by fatigue. Exercise raises blood glucose levels because the primary energy source for glycolysis and oxidative phosphorylation is tissue glycogen from the muscle and liver (Cairns, 2006). High amounts of liver and muscle glycogen may lengthen endurance periods and performance during intense exercise while decreasing tiredness (Torma *et al*., 2019). The amount of tissue glycogen may be increased by substances with anti-fatigue properties. The hepatic and muscle glycogen levels as well as the endurance swimming time, were all raised in mice treated with *Glossogyne tenuifolia* extract (Chen *et al*., 2022). In our present study, liver glycogen levels were increased, and serum lactic acid levels were decreased in the animals treated with SPE suggesting its anti-fatigue effect.

To conclude, feeding sheep placental extract improves learning, memory, and exercise performance in ageing mice.

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*IRB approval*

All animal treatment procedures were performed in accordance with the Guide for Care and Use of Laboratory

Animals (National Institutes of Health Publication NO 85-23, raised 1996).

*Ethical statemnent*

Animal-experiment procedures in the present study were approved by the Institutional Animal Care and Use Committee (IACUC) of Providence University (Approval No: 20201218 A008).

*Statement of conflict of interest*

The authors have declared no conflict of interest.

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