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Edible Bird's Nest Soup (EBNS) Serves as Anti-Obesity and Antilipemic After 6-Weeks of Supplementation in High-Fat Diet (HFD)-Fed Rats

(Sup Sarang Burung Walit (EBNS) Berfungsi sebagai Anti-Obesiti dan Antilipemik pada Tikus Selepas 6 Minggu Pemberian Makanan Diet Tinggi Lemak (HFD))

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ABSTRACT

Traditionally, edible bird's nest (EBN) has been trusted to have several medicinal effects in the Chinese community. One of the beneficial effects of the EBN is that it is a metabolic stimulator. This study was conducted to investigate the effect of EBN soup (EBNS) and EBN extract (EBNE) on obesity, body fat distribution, blood lipid profile, and cardiogenic indices in high-fat diet-fed rats. Obesity and dyslipidemia in rats were induced with a high-fat diet (HFD) and Triton X-100 (TX100) and supplemented with or without Simvastatin, EBNS, or EBNE for 6-weeks. Final body weight changes and body fat distribution (subcutaneous and visceral fat) were measured. Triglycerides (TAG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were measured by taking blood from the lateral coccygeal vein. Subsequently, cardiogenic indices were calculated (LDL/HDL ratio (LHR) and atherogenic index (AI)). In general, the EBNS demonstrated an anti-obesity and antilipemic effect after 6 weeks of supplementation. A significant reduction in body fat deposition was also recorded in the EBNS group, including subcutaneous and visceral fat. Bad cholesterol (LDL) was significantly maintained in the normal range, and cardioprotectant cholesterol (HDL) was significantly elevated as good as Simvastatin. EBNS supplementation was also significantly predicted to reduce the risk of cardiovascular diseases and atherosclerosis development in the rat model. Nonetheless, the EBNE group was not exhibiting equivalent effects as documented in the EBNS group after 6 weeks of supplementation.

Keywords: Atherosclerosis; cardiovascular diseases (CVD); dyslipidemia; edible bird's nest (EBN); obesity

ABSTRAK

Secara tradisi, sarang burung walit (EBN) dipercayai oleh masyarakat Cina untuk memberikan beberapa kesan perubatan. Salah satu khasiat perubatan EBN adalah untuk bertindak sebagai penggalak metabolik. Penyelidikan ini dijalankan bagi mengkaji kesan sup EBN (EBNS) dan ekstrak EBN (EBNE) dalam obesiti, taburan lemak badan, profil lipid darah dan indeks kardiogenik di dalam tikus yang diberi makan diet tinggi lemak. Obesiti dan dislipidemia pada tikus telah diaruhkan melalui diet tinggi lemak dan Triton-X 100 (TX100) dan tambahan juga dengan atau tanpa Simvastatin, EBNS atau EBNE selama 6 minggu. Perubahan berat badan akhir dan taburan lemak badan (lemak subkutaneus dan viseral) telah diukur. Trigliserida (TAG), jumlah kolestrol (TC), lipoprotein ketumpatan-tinggi (HDL), lipoprotein ketumpatan-rendah (LDL) dan lipoprotein ketumpatan-sangat rendah (VLDL) telah dihitung melalui darah yang diambil daripada vena koksigeal. Seterusnya, indeks kardiogenik juga telah dihitung (nisbah LDL/HDL (LHR) dan indeks aterogenik (AI)) di dalam kajian ini. Secara umumnya, EBNS telah menunjukkan kesan anti-obesiti dan antilipemik selepas diambil secara tambahan selama 6 minggu. Penurunan taburan lemak badan secara signifikan di dalam kumpulan EBNS telah direkodkan melalui pengumpulan lemak subkutaneus dan viseral. Kolesterol jahat (LDL) telah berjaya dikekalkan secara signifikan di dalam julat normal dan kolesterol kardioperlindung (HDL) menunjukkan peningkatan yang signifikan yang setanding dengan Simvastatin. Tambahan EBNS juga dijangka secara signifikan untuk mengurangkan risiko pembentukan penyakit

kardiovaskular dan aterosklerosis dalam model tikus ini. Walau bagaimanapun, kumpulan EBNE tidak menunjukkan kesan yang setara dengan EBNS selepas ia diberi secara tambahan selama 6 minggu.

Kata kunci: Aterosklerosis; dislipidemia; obesiti; penyakit kardiovaskular (CVD); sarang burung walit (EBN)

INTRODUCTION

Based on the World Health Organisation (WHO), approximately 34% of the world's population dies annually due to cardiovascular disease (CVD), and this figure is equivalent to 17.9 million deaths per year. In 2022, a total of RM9.65 billion spent by the Malaysian government on preventing and managing CVD (MOH 2022). Epidemiologically, atherogenic dyslipidemia and obesity are among the common causes linked to the development of CVD (Chan, Barrett & Watts 2014; Powel-Willey et al. 2021). For the past few decades, ethnomedical researchers have focused on plant-based natural products, and limited studies have been conducted related to animal-based natural products (Andriamanantena et al. 2023).

One of the animal-based natural products that Chinese people typically consume for health reasons is edible bird's nest (EBN). It had been derived from the salivary secretion of the swiftlet (*Aerodramus fuciphagus*). EBN was well documented in Traditional Chinese Medicine (TCM) books and traditionally prepared in a tonic soup (Marcone 2005). Haghani et al. (2016) have documented their medicinal benefits, including energy and metabolic stimulants.

In general, EBN is mainly composed of proteins (54.0–63.0%) (Liu et al. 2012; Zulkifli et al. 2019). Since the primary component of EBN is a protein, most of the extraction methods in previous studies aimed to extract the protein in an aqueous form via boiling at various temperatures (70-100 °C) (Utomo et al. 2014; Zulkefli, Chua & Rahmat; Zulkifli et al. 2019). Various studies were conducted to characterise the main bioactive ingredients of this natural product, and almost all studies showed variable results due to the different methods of extraction that were applied in the respective studies (Zulkefli, Chua & Rahmat 2017). However, majority of the studies identified sialic acid as the major constituent to be found in the EBN (Liu et al. 2012; Mohamad Nasir et al. 2021).

In our study, we focused on the medicinal effects of the EBN on obesity, subcutaneous fat, and visceral fat deposition. Concurrently, regulating blood lipid profiles and cardiogenic indices as early as 6 weeks of treatment. Therefore, we hypothesised that two types of EBN preparations, including the EBN soup (EBNS) and EBN extract (EBNE), were able to ameliorate obesity, subcutaneous fat thickness, visceral fat accumulation, blood lipid profile, and cardiogenic indices in the HFD-fed rats after six weeks of consumption.

MATERIALS AND METHODS

ANIMAL STUDY AND EXPERIMENTAL DESIGN

A total of 30 (N) male Sprague-Dawley rats aged 10-weeks old were obtained from the Animal Research Facility (ARF), Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM). Upon arrival, all rats were kept at the Non-Infectious Research Centre of Veterinary Medicine for two weeks of acclimatisation. They were kept in a controlled environment with an equal light cycle (12 h of dark and light photoperiods). All procedures and experimental designs were approved by the Institution Animal Care and Use Committee (IACUC), Research Management Centre (RMC) of UPM (UPM/IACUC/AUP-R084/2017).

Post-acclimatisation, they were assigned to five different groups of treatment, including baseline control (BC), negative control (NC), positive control (PC), EBNS, and EBNE (n = 6). BC was fed with a normal rat diet (Cargill, Malaysia), while NC was fed via spontaneous feeding of a high-fat diet (HFD) [55% (Fat) and 1.25% (Cholesterol)] (TD. 02028, Envigo, UK) and parenterally administered with a single dose of Triton X-100 on Day 0 (150 mg/kg, SQ, SID) (Sigma-Aldrich, Germany) for the obesity and dyslipidemia induction. The PC is similar to the NC with additional treatment with Simvastatin (10 mg/kg, PO, SID) as an anti-cholesterol drug (Zocor 40 mg, Merck Sharp & Dohme (MSD), USA). Similar to the EBNs group, obesity and dyslipidemia were induced and simultaneously supplemented with EBNS (843.2 mg/kg) and EBNE (6.5 mg/kg), respectively, via oral gavage on a daily basis. The dosage for EBNS was derived from a human conversion dosage based on Traditional Chinese Medicine (TCM) formulation (Xiaosheng 2011) by using Animal Equivalent Dosage (AED) from the Food and Drug Administration (FDA). Meanwhile, EBNE was calculated based on the safe and effective dosage of the in vitro study using the in vivo conversion formula from FDA guidelines (Akmal et al. 2020). Treatment was conducted for six weeks (Figure 1).

EBN SOUP AND EXTRACT PREPARATION

Raw, unclean EBN (RU EBN) was purchased from a certified collector (U-Le Bird Nest Trading, Perak, Malaysia). RU EBN was manually cleaned before being ground into small granules. EBNS was prepared freshly before supplementation by stewing the EBN granule with PBS in a conical tube at a ratio of 100 mg of EBN granule

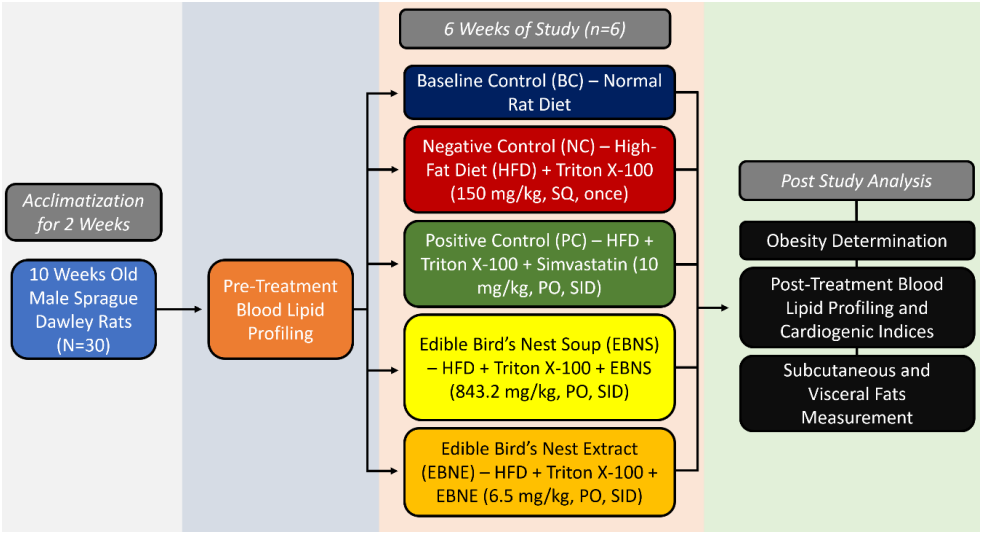

FIGURE 1. A brief study design depicted in a flowchart. Baseline control (BC); Negative Control (NC); Positive Control (PC); EBN Soup (EBNS); EBN Extract (EBNE); SQ (subcutaneous); PO (orally); SID (once a day)

to 1.5 mL of PBS. The EBN was soaked overnight before stewing in the water bath at 70 °C for 4 h. Cooled EBNS was transferred into a sterile syringe before oral gavage (843.2 mg/kg) to the rats on a daily basis. Similarly, the EBN granule was also used for the EBNE preparation. Each gram of EBN was stewed in the same manner in 20 mL of PBS. Post-stewing, only the soluble part of EBN was withdrawn and subjected to the protein precipitation technique (cold acetone precipitation). Solubilised protein pallet was quantified (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific, USA) before being supplemented (6.5 mg/kg) to the rats on a daily basis.

OBESITY DETERMINATION

Prior to the study's commencement, the initial body weight was recorded. After 6 weeks of the study, the final body weight was documented again before the euthanasia. This final body is crucial for dosage determination during euthanasia and will also be used for obesity determination. Based on Woods et al. (2003), obesity in rats can be determined when the final body weight exceeds a 10% increment compared to the control group fed with a standard chow diet.

BLOOD LIPID PROFILING AND CARDIOGENIC INDICES

Post-acclimatisation, pre-treatment blood was collected before the study was conducted, and after week 6 of treatment, blood was collected again for blood lipid profiling. Blood collection was performed using 25G needles attached to the 1 mL syringe, and withdrawal was done via the lateral coccygeal vein. The collected whole blood in the plain red tube was kept at 4 °C before being centrifuged at 10,000 rpm for 10 min. The serum was collected using a micropipette and subjected to the blood lipid profile, including total triglycerides (TAG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL). Collected blood serums were subjected to a colorimetric quantitative ELISA assay according to the manufacturer's user manual (Triglycerides, Cholesterol, and HDL Liquicolor, Human Diagnostics Worldwide, Germany; and Rat LDL and VLDL ELISA Kit, Elabscience, USA). Cardiogenic indices, including the LDL/HDL Ratio (LHR) and Atherogenic Index (AI), were calculated to predict the risk of cardiovascular diseases (CVD) and atherosclerosis incidence, respectively, using the following formulas:

$$\text{LHR} = \text{Low-Density Lipoprotein} / \text{High-Density}$$

$$\text{LipoproteinAI} = (\text{Total Cholesterol} - \text{High-Density}$$

$$\text{Lipoprotein}) / \text{High-Density Lipoprotein}$$

SUBCUTANEOUS FAT THICKNESS AND VISCERAL FAT MEASUREMENT

After the study was completed, rats were euthanized using an overdose of Pentobarbital (18%) at 200 mg/kg intraperitoneally (IP). Upon dissection, the subcutaneous

fat thickness and visceral fat weight were recorded. Subcutaneous fat was measured at Lumbar 1- Lumbar 5 (L1–L5) vertebrae using a digital Vernier calliper with three technical replicates for each rat in millimetres (mm). Meanwhile, the visceral fat was harvested from the abdominal cavity and weighed using the digital balancer to get the total amount of the visceral fat in grammes (g).

STATISTICAL ANALYSIS

All cumulative data were expressed in mean ± standard deviation (SD). The statistical difference between the groups was calculated via one-way ANOVA and validated with Tukey's HSD post hoc test ($p<0.05$), using IBM Statistical SPSS 26.0 (Chicago, USA).

RESULTS

OBESITY DETERMINATION

Initial body weight in Table 1 showed no significant difference among the groups. After six weeks of treatment, the NC (477.34 ± 33.16), PC (468.48 ± 45.76), and EBNE (451.32 ± 21.16) groups were statistically showing significant body weight increments compared to the BC (398.31 ± 29.05) group. Meanwhile, the EBNS (417.30 ± 34.80) group had shown no significant body weight increment compared to the BC group. In addition, the NC, PC, and EBNE documented 19.84%, 17.62%, and 13.31% body weight increments, respectively, compared to the BC.

BLOOD LIPID PROFILING

In the beginning, prior to the treatment being conducted (Week 0), all the lipid parameters, including triglycerides (TAG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), were measured. Based on the data tabulation in Table 2, there was an insignificant difference between the treatment groups, and all data were homogenously distributed.

Based on the data in Table 3, the NC group was showing significant elevations of TAG (4.90 ± 0.51), TC (11.28 ± 0.89), LDL (26.52 ± 0.50), and VLDL (4.54 ± 0.31) compared to the BC group. Concomitantly, NC was significantly showing a reduction of good cholesterol, HDL (2.08 ± 0.04), after 6 weeks of treatment. In contrast, the Simvastatin-treated group (PC) was showing significant reductions in TAG (1.40 ± 0.97), TC (5.61 ± 0.67), LDL (1.42 ± 0.12), and VLDL (1.10 ± 0.30) compared to the NC group. Concurrently, the HDL (4.60 ± 0.64) in the PC group was significantly elevated, up to 74% and 121%, respectively, compared to the BC and NC groups. Meanwhile, the EBNS-treated group was demonstrating a similar trend of effects as the PC group, with reductions in TAG (1.53 ± 0.57), TC (6.99 ± 0.77), LDL (1.39 ± 0.25), and VLDL (1.60 ± 0.18). Statistically, the EBNS group was observed to regulate blood lipid profiling up to the normal reading as measured in the BC group for TAG (1.75 ± 0.30), LDL (2.07 ± 0.04), and VLDL (1.74 ± 0.06) parameters. Cardioprotectant lipid in the EBNS (5.34 ± 0.64) group was showing a significant 157% elevation compared to the NC and was significantly higher than the BC (2.64 ± 0.30) group, up to 102%. Similarly, the EBNE was able to statistically reduce the lipid parameters, including TAG (2.74 ± 0.37), TC (9.87 ± 0.77), LDL (10.56 ± 0.33), and VLDL (2.00 ± 0.08), compared to the NC group, but not statistically reduce them in comparison to the BC group. Meanwhile, the cardioprotectant lipid, HDL (3.48 ± 0.99), was showing significant elevation compared to the NC group and remained statistically equivalent compared to the BC group.

LDL/HDL RATIO (LHR) AND ATHEROGENIC INDEX (AI)

The LDL/HDL ratio (LHR) was calculated based on blood lipid profiling to predict the risk of CVD, and the protective ratio should be lower than 3.0 (Table 4). Only BC (0.79 ± 0.08), PC (0.31 ± 0.06), and EBNS (0.26 ± 0.04) were calculated to be less than 3.0 and considered to be protected from cardiovascular diseases. Simultaneously, both ratios for the PC and EBNS showed no significant difference. Meanwhile, the NC (12.76 ± 0.14) was showing the highest LHR and was significantly higher than the other groups. On the other hand, the EBNE (3.22 ± 0.84) group was significantly reduced compared to the NC, but the value was not in the protective range.

AI was calculated to predict the risk of atherosclerosis, and the protective index should be lower than 1.6 (Table 4). Only BC (0.86 ± 0.16), PC (0.35 ± 0.30), and EBNS (0.33 ± 0.23) were calculated lower than the 1.6 ratio. As expected, the NC (4.43 ± 0.49) group recorded more than a protective ration. In addition, the EBNE (2.03 ± 0.88) group also showed a significantly spiked risk of atherosclerosis index with a ratio greater than 1.6.

SUBCUTANEOUS FAT THICKNESS AND VISCERAL FAT MEASUREMENT

Table 5 tabulates subcutaneous fat thickness and visceral fat measurements after 6 weeks of study. Based on this table, the NC (15.20 ± 0.66) and EBNE (14.27 ± 0.15) groups showed significant elevations in subcutaneous fat thickness compared to the BC (12.58 ± 0.28) group. Meanwhile, the PC (13.84 ± 0.21) and EBNS (11.73 ± 0.15) recorded normal subcutaneous fat thickness compared to

TABLE 1. The initial and final body weights of the rats after 6 weeks of study are expressed in mean ± standard deviation. The percentage of body weight increment is also stated in the last column of the table. Different alphabets are showing significant differences compared to baselinecontrol (BC). Negative Control (NC); Positive Control (PC); EBN Soup (EBNS); EBN Extract (EBNE)

Groups	Initial body weight (g)	Final body weight (g)	Body weight increment (%)
BC	290.11 ± 31.40[a]	398.31 ± 29.05[a]	0.00
NC	277.26 ± 43.11[a]	477.34 ± 33.16[b]	19.84
PC	298.02 ± 39.41[a]	468.48 ± 45.76[b]	17.62
EBNS	297.67 ± 25.59[a]	417.30 ± 34.80[a]	4.76
EBNE	303.34 ± 16.53[a]	451.32 ± 21.16[b]	13.31

TABLE 2. Pre-treatment blood lipid profiling in the respective treatment groups and expressed in mean ± standard deviation. Similar alphabet is showing an insignificant difference compared to baseline control (BC). Negative Control (NC); Positive Control (PC); EBN Soup (EBNS); EBN Extract (EBNE)

Group	TAG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)
BC	2.19 ± 0.38[a]	4.04 ± 0.44[a]	4.21 ± 0.77[a]	1.96 ± 0.24[a]	1.67 ± 0.21[a]
NC	2.19 ± 0.56[a]	3.75 ± 0.64[a]	4.58 ± 0.74[a]	1.38 ± 0.15[a]	1.99 ± 0.24[a]
PC	2.31 ± 1.03[a]	4.16 ± 0.48[a]	4.33 ± 0.36[a]	1.42 ± 0.12[a]	1.74 ± 0.07[a]
EBNS	2.17 ± 0.22[a]	3.00 ± 0.82[a]	4.71 ± 0.45[a]	1.76 ± 0.12[a]	1.74 ± 0.15[a]
EBNE	2.20 ± 0.38[a]	3.98 ± 0.36[a]	4.96 ± 0.39[a]	1.08 ± 0.14[a]	1.73 ± 0.33[a]

TABLE 3. Post-treatment blood lipid profiling in the respective treatment groups is expressed in mean ± standard deviation. Similar alphabet is showing an insignificant difference compared to baseline control (BC). Negative Control (NC); Positive Control (PC); EBN Soup (EBNS); EBN Extract (EBNE)

Group	TAG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)
BC	1.75 ± 0.30[a]	5.23 ± 0.97[a]	2.64 ± 0.30[a]	2.07 ± 0.04[a]	1.74 ± 0.06[a]
NC	4.90 ± 0.51[b]	11.28 ± 0.89[b]	2.08 ± 0.04[b]	26.52 ± 0.50[b]	4.54 ± 0.31[b]
PC	1.40 ± 0.97[a]	5.61 ± 0.67[a]	4.60 ± 0.64[c]	1.42 ± 0.12[a]	1.10 ± 0.30[c]
EBNS	1.53 ± 0.57[a]	6.99 ± 0.77[c]	5.34 ± 0.64[d]	1.39 ± 0.25[a]	1.60 ± 0.18[a]
EBNE	2.74 ± 0.37[c]	9.87 ± 0.77[d]	3.48 ± 0.99[a]	4.56 ± 0.33[c]	2.00 ± 0.08[a]

TABLE 4. Calculated LDL/HDL ratio (LHR) and atherogenic index (AI) for each respective group after 6 weeks of treatment and expressed in mean ± standard deviation. Different alphabets are showing significant differences compared to BC. Baseline Control (BC); Negative Control (NC); Positive Control (PC); EBN Soup (EBNS); EBN Extract (EBNE)

Treatment group	LDL/HDL Ratio (LHR)	Atherogenic Index (AI)
BC	0.79 ± 0.08^{a}	0.86 ± 0.16^{a}
NC	12.76 ± 0.14^{b}	4.43 ± 0.49^{b}
PC	0.31 ± 0.06^{c}	0.35 ± 0.30^{c}
EBNS	0.26 ± 0.04^{c}	0.33 ± 0.23^{c}
EBNE	3.22 ± 0.84^{d}	2.03 ± 0.88^{d}

TABLE 5. Post-study measurement of the subcutaneous fat thickness (mm) and visceral fat (g) in the respective group expressed in mean ± standard deviation. Different alphabets are showing significant differences compared to BC. Baseline Control (BC); Negative Control (NC); Positive Control (PC); EBN Soup (EBNS); EBN Extract (EBNE)

Treatment group	Subcutaneous fat thickness (mm)	Min-Max (mm)	Visceral fat (g)	Min-Max(g)
BC	12.58 ± 0.28^{a}	11.9-12.9	15.30 ± 0.46^{a}	14.61-15.84
NC	15.20 ± 0.66^{b}	14.2-16.0	42.97 ± 2.08^{b}	40.67-45.83
PC	13.84 ± 0.21^{a}	13.4-14.2	28.21 ± 0.51^{c}	28.23-29.05
EBNS	11.73 ± 0.15^{a}	11.6-12.1	14.49 ± 0.23^{a}	14.24-14.85
EBNE	14.27 ± 0.15^{c}	12.4-14.4	16.82 ± 1.04^{d}	15.22-17.68

the BC group. On the other hand, only the EBNS (14.49 ± 0.23) group documented normal visceral fat content in the abdominal cavity compared to the BC (15.30 ± 0.46) group. Meanwhile, NC (42.97 ± 2.08), PC (28.21 ± 0.51), and EBNE (16.82 ± 1.04) were exhibiting significant elevations of visceral fat accumulation in the abdominal cavity compared to BC. However, the PC and EBNE groups significantly reduced the visceral fat content when compared to the NC group.

DISCUSSION

Obesity and atherogenic dyslipidemia are among the key risk factors that can contribute to the occurrence of metabolic syndrome (MetS). One of the disease components constituting this syndrome is CVD. It is epidemiologically and clinically proven that obesity and atherogenic dyslipidemia are linked to the occurrence of CVD. This disease is one of the economic burdens in developed and developing countries, as the government has to endure the treatment costs and human capital lost each year (Chan, Barrett & Watts 2014; MOH 2022). In the National Health and Morbidity Survey 2019, approximately 43.3% and 17.7% of the Malaysian population were diagnosed with atherogenic dyslipidemia and obesity, respectively. Therefore, effective modality in preventing and managing this disease is one of the cores of the Malaysian government's policy in the National Strategic Plan for Non-Communicable Disease (NSP-NCD) 2016-2025 (MOH 2022).

In 2021, Malaysia produced 478.23 metric tonnes of RU EBN and exported it to the international market for RM 1.112 billion. Constant and sustainable production of the EBN in Malaysia serves as a privilege for Malaysian dietary consumption and supplementation (DVS 2022). In the current study, we have designed an experiment to provide insights for prophylactic purposes after 6 weeks of EBN consumption, with the induction of dyslipidemia and obesity (HFD and Triton-X) using male Sprague Dawley rats concurrently with the supplementation of EBN. Male rats were utilised in this study as no influences of physiological changes due to the oestrous cycle in rats that could be altered the response of the rats on the dyslipidemia induction and supplementary intake (Lindhardt et al. 2022). This experimental design is also consistent with the traditional use of EBN as a food supplement that is commonly consumed by the Chinese community. The current dosage of the EBNS is based on the human's

average consumption conversion, and this dosage is less than 10% of the daily value (DV) for the feed supplement as defined by the FDA (2020). Meanwhile, EBNE dosage is an *in-vitro* dosage conversion based on the effective dosage of the extract, as reported by Akmal et al. (2020).

In the development of obesity, there are two important parameters of body fat distribution that have to be taken into consideration, including subcutaneous and visceral fat (Mandarim-de-Lacerda et al. 2021). In an obese rat model, these two parameters will be elevated compared to a normal rat model due to adipocyte hyperplasia and hypertrophy as a result of excessive calorie intake (Chusyd et al. 2016; Kolb 2022). This finding is parallel with our result in the NC group that had been fed with HFD, which demonstrated significant elevation of subcutaneous and visceral fat deposition in our rat model. Despite high influx of calories from the HFD, simultaneous oral supplementation of the EBNS was able to maintain these two parameters at the normal range compared to the BC, which reflected that EBNS supplementation was able to prevent fat deposition in the subcutaneous tissue and abdominal cavity.

Constant spontaneous feeding of the HFD was able to cause obesity in the NC group, with body weight increments exceeding 10% of the average BC group body weight. On the other hand, the Simvastatin-treated group was not able to maintain normal body weight and was classified as an obese group. This finding is consistent with a previous study that mentioned that the statin drug group only efficiently manages abnormal blood lipid profiles but not obesity (Sharpton et al. 2017). In supplementation of the EBNS during spontaneous HFD feeding in the rat model, the EBNS group showed an anti-obesity effect with less than 10% body increment compared to the average body weight of the BC group, and this finding is consistent with the study by Woods et al. (2003). Besides that, a similar finding had been reported by Yida et al. (2015), who mentioned that the EBN has an effect on regulating body weight. Contrary to this, a recent study conducted by the same researcher (Yida et al. 2022) discovered the EBN had no effect on body weight management. To the best of our knowledge, the EBN had been identified as one of the natural products that contained a high amount of sialic acid as the major bioactive compound (Liu et al. 2012), and a study conducted by Peng et al. (2019) demonstrated that the sialic acid was able to prevent the occurrence of obesity in the animal model, particularly in rodent species. Thus, this might explain the reason for the EBN's effect on regulating body weight in the dietary-induced obesity rat model. Furthermore, we can clarify that the EBN regulates body weight by limiting the deposition of fat in the subcutaneous tissue layer and visceral organs.

Dyslipidemia is a condition characterised by the elevation of blood TAG, TC, LDL, and VLDL, coupled with the depletion of good cholesterol, HDL (Chan, Barrett & Watts 2014; Powel-Willey et al. 2021; MOH 2022). As the rat was constantly fed with HFD and parenterally administered with Triton X, the NC group demonstrated a dyslipidemic blood lipid profile with significant elevations of TAG, TC, LDL, and VLDL and a reduction of HDL compared to the BC group. The simultaneous supplementation of EBNS to the rats in the EBNS group showed a normal blood lipid profile. This finding reflects that the EBNS is able to prevent the development of dyslipidemia, and this might suggest a similar effect when humans start to consume the EBNS consistently. In the EBNS group, TC was significantly elevated compared to the BC group. This is because HDL, which protects the heart, is significantly higher in the EBNS group than in any other group. HDL is also counted as one of the sub-cluster cholesterols in the TC calculation, along with LDL and TAG (MOH 2019). On the other hand, the EBNS supplementation also significantly maintained bad cholesterol at a normal range compared to the BC group. Based on the blood lipid profile, the EBNS had a relatively similar effect to the anti-cholesterol drug (Simvastatin) in the PC group. This finding is consistent with a study conducted by Akmal et al. (2020) and Yida et al. (2022), in which they mentioned that the EBN is able to upregulate the LDL receptor (LDLR) via suppression of the rate-limiting cholesterol metabolism enzyme, HMGCR. Thus, the expression of the LDLR in the body tissue, particularly in the liver, will increase the reuptake of excess LDL circulating in the blood into the tissue (Chan, Barrett & Watts 2014; Powel-Willey et al. 2021).

Chronic dyslipidemia could lead to the development of CVD. Currently, the most efficient medical intervention in preventing and managing this condition is the constant taking of statin drugs, including Simvastatin (Pappan & Rehman 2022). This drug had been administered to the rats in the PC group, and it demonstrated a protective ration in the LHR. In contrast, constant consumption of HFD without the anti-cholesterol drug as implemented in the NC group showed unprotective LHR and might expose it to the development of CVD. Upon supplementation with EBNS, the LHR was maintaining its protective range and diminishing the risk of CVD development. This is due to the significant elevation of cardioprotectant lipoprotein (HDL) in the current study. This HDL is able to translocate excess cholesterol in the liver for excretion via reverse cholesterol transport (RCT) and concurrently mediate neutralising oxidative stress due to reactive oxygen species (ROS) in the cardiomyocytes (Nagao et al. 2018). In order to predict the occurrence of atherosclerotic cardiovascular disease (ASCVD) in our rat model, AI was calculated, and the EBNS group recorded a protective index that was statistically equivalent to the Simvastatin-treated (PC) group. This protective index is highly associated with

anti-obesity and anti-lipemic effects, as documented in the current study, because these two effects are able to reduce the occurrence of CVD and ASCVD (Chan, Barrett & Watts 2014; MOH 2019; Pappan & Rehman 2022). This finding is also consistent with the study conducted by Akmal et al. (2018), which demonstrated the EBNS ability to reduce atherosclerosis in the major blood vessels.

In general, the EBNE group was not exhibiting equivalent effects compared to the EBNS group as an anti-obesity and anti-lipemic agent. We have also postulated that the amount of bioactive ingredient (sialic acid) in the extract was not sufficiently available to give the desired effect compared to EBNS, which may be due to enzymatic and metabolic processes in the gastrointestinal tract (Octava et al. 2015). This statement is in parallel with findings from several proteomic and metabolomics studies that have mentioned that the bioactive ingredient of EBN is persistently incorporated in the gelatinous and insoluble parts of the EBN that are only available in EBNS (Chua et al. 2014; Utomo et al. 2014; Zulkefli, Chua & Rahmat 2017).

CONCLUSION

In the present study, the authors have briefly concluded that the EBNS has an anti-obesity and anti-lipidemic property in HFD-fed rats after 6 weeks of supplementation. The effects of two different types of EBNs are varied. The EBNS has profound effects compared to the EBNE in ameliorating body weight management and blood lipid profiles. The lipoprotein parameters also showed the EBNS had a greater effect compared to the EBNE and were statistically comparable with the Simvastatin-treated (PC) group. Therefore, consumption of 1-3 EBN per serving for 2-4 times a week (Xiaosheng 2011), could potentially improve body weight management, blood lipid profile, and cardiogenic indices.

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