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ORIGINAL ARTICLE

The Effects of *Elateriospermum tapos* against Obese Maternal Rat in Mitigating Obesity Development among their Adult Female Offspring

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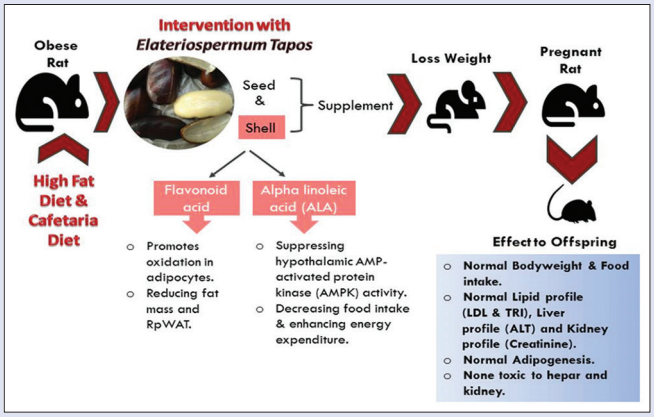
ABSTRACT

Background: Maternal obesity is a metabolic disorder described by chronic inflammation and dyslipidemia and influences non-communicable diseases in offspring. *Elateriospermum tapos* is a plant containing high alpha-linolenic acid with antiobesity effect. **Objectives:** This study was aim to investigate the effect of *E. tapos* supplementation in obese rat prior pregnancy on the female offspring's body weight, liver and kidney profile, and histological changes of heart, liver, kidney, and retroperitoneal white adipose tissue (RpWAT). **Materials and Methods:** Thirty female Sprague Dawley rats were used in this study. Six rats were assigned to dams normal diet group fed with standard chow diet. The remaining rats were fed with high-fat and cafeteria diet (HFCD) to generate obesity for 5 weeks. The obese rats were further divided into 4 groups; group1; Dams Negative Control (DNC, normal saline only), group 2; Dams Positive Control (DPC, Orlistat drug 200mg/kg), Dams Treatment 1 (DTX1, *E. tapos* seed 200 mg/kg) and Dams Treatment 2 (DTX2, *E. tapos* shell 200mg/kg) extracts for 6 weeks daily prior to mating. The weaning offspring were then designated into 6 groups according to their dam's group (n = 6/group). The offspring were culled at 12th week of age in order to have their blood sample and organs. **Results:** Adult offspring from DTX2 group showed a decrease in weight gain, alanine aminotransferase, and creatinine level compared to adult offspring from DNC. Histological examination showed that liver and RpWAT of offspring from DTX1 and DTX2 groups were comparable with normal diet group, indicating that the extracts were capable in reversing the high fat diet effect to the organ. **Conclusion:** *E. tapos* shell supplementation was effective in reducing the development of obesity with no harmful effect among adult female rat offspring.

Key words: Animal study, cafeteria diet, childhood obesity, high-fat diet, maternal obesity

SUMMARY

- *Elateriospermum tapos* extraction seed and shell showed no side impacts in liver, heart, kidney, and adipose tissues of rat
- Hot aqueous extraction of seed and shell of *E. tapos* has powerful antiobesity activities to reduce maternal obesity in descendants of female rats
- ALA in *E. tapos* helps decrease the calorie intake of obese dams as well as their first generation and reduces fat accumulation by suppressing the activation of AMP-activated protein kinase activity in the hypothalamus, thus, causes the reduction in body weigh.



Abbreviations Used: ALA: Alpha-Linolenic Acid; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; DNC: Dams negative control; DND: Dams normal diet; DPC: Dams positive control; DTX1: Dams treatment 1 (*E. tapos* seed); DTX2: Dams treatment 1 (*E. tapos* shell); HFCD: High fat cafeteria diet; NBF: Neutral buffered formalin; NCD: Non-communicable disease; OCD: Offspring cafeteria diet; ONC: Offspring negative control; OND: Offspring normal diet; OPC: Offspring positive control; OTX1: Offspring treatment 1 (*E. tapos* seed); OTX2: Offspring treatment 1 (*E.tapos* shell); RpWAT: Retroperitoneal white adipose tissue.

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INTRODUCTION

Obesity is currently a crucial global medical problem as it is related to serious complications from non-communicable diseases such as cardiovascular diseases, type two diabetes, and various types of cancers.[1] The global obesity prevalence continues increasing unabatedly with 2.7 billion adults are predictable to be overweight or have obesity

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by 2025.An obesity rate differs particularly across South East Asia, with Malaysia recording amongst the highest levels. In Malaysia, prevalence of obesity among female is higher compared to male (11.4% in males; 16.7% in females).

Global Nutrition Study 2017 found that 54% of overweight or obese women are of childbearing age.[2] Excessive gestational weight gain and subtle changes in behavior or lifestyle, such as increased calorie intake, a less balanced diet and decreased postpartum physical activity, are two key factors that may lead to overweight or obesity throughout childbearing years. Maternal obesity is at higher risk of having a baby that is large for gestational age and overweight or obese in childhood.[3] Obesity among children has becoming serious global issues as the prevalence was increasing every year.[4] In 2016, without a doubt the number of obese children wherever all through the world were assessed to be 124 million. High BMI in early ages regularly causes the development of chronic diseases in adulthood such as cardiovascular diseases and type II diabetes.[5]

All these insights above indicate why an extensive multi-level solution to childhood obesity needs to be put forward for future generations to achieve a healthier lifestyle. It is therefore essential to treat obesity as it is the cause of many illnesses. The prevalent side effects of conventional medication for obesity such as headache, constipation, heart arrhythmia, and more diseases have contributed to an increased use of traditional herbal medicine.[6] Consequently, procedures using comparatively secure and less side effects of natural products are gaining interest in curing obesity.

Elateriospermum tapos is a species of plant of *Euphorbiaceace* family and are classified into subfamily of *Crotonoideae* and *Elateriospermeae* tribe. Widely found in Southeast Asian tropical rainforest which includes peninsular Malaysia, peninsular Thailand, Brunei, Sumatra, Java, and Borneo. *E. tapos* commonly applied in wound healing treatment[7] and treating chronic diseases such as hypertension.[8] The preliminary study on *E. tapos* seed and shell extracted with hot water extraction obtained the highest phenolic and flavonoid content and exhibited the best pancreatic lipase and antioxidant activity.[9] Observing the beneficial association between phenolic and flavonoid compounds and free radical scavenging and antilipase activity indicated that these compounds account for the antiobesity and antioxidant effect of *E. tapos* extraction. Study on the antiobesity effect among lean and high-fat and cafeteria diet (HFCD) female rat has been proven that *E. tapos* seed and shell exhibited the antiobesity effect with the treatment prior the obesity induction showed better result compared to treatment of post obesity induction.[10] The current study was to evaluate the effect of *E. tapos* seed and shell treatment prior pregnancy of obese rat to their first-generation specifically their female adult offspring at the 12th week of age. The body/organ weight, calorie intake, liver and kidney function test, and also histological changes of the vital organ among the first generation of research rats were studied.

MATERIALS AND METHODS

Plant material and extraction

Fresh fruit of *E. tapos* from Jengka, Pahang, Malaysia, were collected. Voucher specimens of *E. tapos* (UPMSK3154117) were deposited at the Institute of Bioscience, University Putra Malaysia. The *E. tapos* fruit was oven dried for overnight (24 h) at temperature of 60°C followed by separation of seeds from the shell then grounded and sieved. Extraction of seed and shell of *E. tapos* was done by using the hot aqueous extraction method modified from Cheurfa and Allem.[11] Fifty grams of pounded *E. tapos* in 1 L Scott bottle wrapped with aluminum foil, were then added to 500 ml of distilled water and put in a water bath at 70°C for 24 h. After 24 h, solution was let cool before it was filtered using Whatman No. 1 filter paper. The resulting viscous material was dried freezing to guarantee full solvent removal and remained at −20°C before feeding it to the rats.

Preparation of high fat diet

The HFD composition for this research was adapted based on Santhra *et al.* and Kokila *et al.* modification.[10,12] HFD contains 414 kcal/100 g consisting of 40% fat, 17% protein, and 43% carbohydrates. Required ingredients are 68% of a commercial rat pellet (Gordon Specialty Stockfeed, Malaysia), 6% of ghee (Crispo), 20% of full cream milk powder (Dutch lady), and 6% of corn oil (Vecorn). All ingredients were then mixed and baked in the Memmert U universal heating oven at 60°C for 2 h. HFD was administered for 5 weeks to obese induced rats to produce obesity on dams.

Experimental design

All animal-related procedures were carried out under the approval of Management and Science University's Animal Care and Use Committee (AE-MSU-073). For this study thirty female Sprague Dawley rats (4–5 weeks old) weighing from 150 to 200 g were used. The rats were housed in plastic boxes (22 cm height × 65 cm length × 40 cm width) with two rats in each box. All rats were acclimatized to 12/12 h light/dark cycle for 1 week in a temperature controlled environment (21°C ± 1°C). The rats were fed with standard chow diet and water was available *ad libitum*.

After acclimatization, the rats were randomly assigned into two groups. Six rats were assigned as dams normal diet group (DND), were fed with commercial rat pellet only, and the rest of the rats were assigned as HFCD group, were fed with commercial rat pellet (306.2 kcal/100 g), high fat diet (HFD) pellet (414 kcal/100 g), and selected cafeteria food including cake (440 kcal/100 g), sausage (260 kcal/100 g), and extruded savory snacks (566 kcal/100 g).

Obesity confirmation was done after 5 weeks by comparing between DND and HFCD groups with significant 15% bodyweight differences. The rats in the HFCD groups were divided randomly into four groups (n = 6/group) as follows; dams negative control (DNC; administered with normal saline), dams positive control (DPC; with 200 mg/kg Orlistat drug supplementation), dams treatment 1 (*E. tapos* seed) (DTX1; with 200 mg/kg *E. tapos* seed supplementation); dams treatment 2 Dams treatment 1 (*E. tapos* shell) (DTX2; 200 mg/kg *E. tapos* shell supplementation). The treatments were provided regularly by oral gavage using a force-feeding needle for 6 weeks while maintaining the HFD and selected food from the cafeteria. To determine the weight gain and calorie intake of rat, body weight and calorie intake were tracked weekly. Mating was performed after 6 weeks of treatment by putting 2 female rats in a cage for 24 h with one normal male rat. The next morning at 8 am, vaginal smears were conducted to test for sperm as a proof of successful mating and this was known as day 0 of gestation. Within 2 days of birth, litter sizes were adjusted to 8–12 pups per dams.

After offspring's weaning phase at post natal day 21 (PND21), they were fed with were designated into 6 groups according to their dam's group (n = 6/group) as follows: offspring normal diet (OND, from DND group); offspring cafeteria diet (OCD from DND group); offspring negative control (ONC from DNC group); offspring positive control (OPC from DPC group); offspring treatment 1 (*E. tapos* seed) (OTX1 from DTX1 group); and offspring treatment 2 (OTX2 from DTX2 group). All offspring groups were fed with standard chow and cafeteria diet except OND which was fed with commercial rat pellet only.

Determination of body weight gain, food intake, and energy intake

Body weight (g) of all rats was recorded weekly using electrical balance. Food consumption (kJ) was determined on a weekly basis by measuring the remaining food and subtracting it from the amount of food provided to achieve the intake of food (kJ).

Collection of plasma, liver, kidney, heart, retroperitoneal white adipose tissue, and visceral fats

Five milliliters blood samples of overnight fasted (12 h) adult female offspring rats (12th weeks of age) were collected in the morning by cardiac puncture under general anesthesia. Each blood sample was collected into heparin tubes and centrifuged at 3500 rpm for 15 min to obtain the plasma. Plasma was collected into a plain tube and stored at −20°C for further biochemical analysis. The rats were then sacrificed by decapitation. Organs/tissue including liver, kidney, heart, retroperitoneal white adipose tissue (RpWAT), and visceral fats were collected and weighed. All organs/tissues were placed at room temperature in 10% neutral buffered formalin (NBF).

Plasma biochemistry

Using Alere Cholestech LDX® Analyzer, parameters of the liver and kidney profile, including alanine aminotransferase (ALT), alkaline phosphatase, and creatinine, were assessed.

Histology studies

The liver, kidney, heart, and RpWAT were collected from the female adult offspring rat (12th weeks of age) and tissue processing proceeded. Once the tissue was sectioned, organs and tissue underwent a series of histology techniques to view the microscopic structure. They were fixed in 10% NBF. It is then embedded and sectioned by the rotary microtome with a thickness of 6 µm. Paraffin ribbon was placed in the water bath at about 40°C–45°C and was transferred onto a glass slide for staining procedures. Sections of the tissue were stained with hematoxylin and eosin. The stained slides were then analyzed using a light microscope, where the tissue sample photomicrographs were captured.

Sudan IV analysis

Sudan IV is used specifically to diagnose atherosclerotic lesion in the arterial tree as a measure of high blood cholesterol presence. This procedure was conducted according to Zhang *et al.*[13] The arterial tree was removed and 'pinned out' on a white wax surface in a dissecting pan using 0.2 mm stainless steel pins. Two dishes were made with 70% ethanol and one dish with 1% Sudan IV solution. The fixed arterial trees were washed for 1 h using running tap water and submerged for 30 min with 70% ethanol. The arterial trees were then placed overnight (12–16 h) face downward in Sudan IV solution at room temperature. After the staining process, the samples were dipped for 3 min into 70% ethanol and then put for 30 min in a fresh 70% ethanol tank. Finally, the samples were washed for 1 h in running tap water before being stored in 10% neutral formalin buffered. The Sudan IV staining is used to highlight the atherosclerotic lesion with dark color and free areas of vessels with clear color.

Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corporation, Armonk, New York). Data has been expressed as mean ± standard error mean. Body weight, calorie intake, organ/tissue weight, and biochemical parameters were analyzed using one-way ANOVA, followed by *post hoc* least significant difference test. Differences were considered significant at $P < 0.05$. The analysis was used to determine the mean difference between the dependent variables.

RESULTS

Effect of *Elateriospermum tapos* supplementation on body weight gain and calorie intake of female offspring

The body weight and food intake of each female offspring rat were recorded on a weekly basis starting from 1st until 12th weeks of age. Both the *E. tapos* seed and shell extraction (200 mg/kg) were used to see if the shell could have the same or better effect as the seed. Distinct body weight separation was observed from 8th weeks onwards. Offspring from HFCD obese dams (ONC) at 8th weeks of age and above shows significant ($P < 0.05$) increase in body weight compared to offspring of rats fed with normal diet (OND). In contrast, body weight of female offspring from obese dams group which was treated with *E. tapos* seed and shell (OTX1 and OTX2) shows significant decrease than ONC [Figure 1]. The lowest total body weight compared to OND group was observed for the OTX2 group followed by the OPC and OTX1 with 16.57%, 9.04% and 1.73% of percentage of body weight difference, respectively [Figure 1].

The HFD groups (OCD and ONC) consumed 26.25% and 26.88%, respectively, higher calorie intake comparative to the OND group. In contrast, the treatment groups (OPC, OTX1, and OTX2) were not significant ($P > 0.05$), but slightly decreased by 15.85%, 18.26%, and 20.66%, respectively compared to HFD group (ONC) [Figure 2].

Effect of *Elateriospermum tapos* on organ and tissue weight of female offspring

The results showed that there was no difference in the weight of organs including liver, kidney, and heart among all tested groups [Table 1]. Meanwhile, the results for total adipose tissues showed that the offspring rats fed cafeteria diet (OCD and ONC) for 12 weeks had significantly

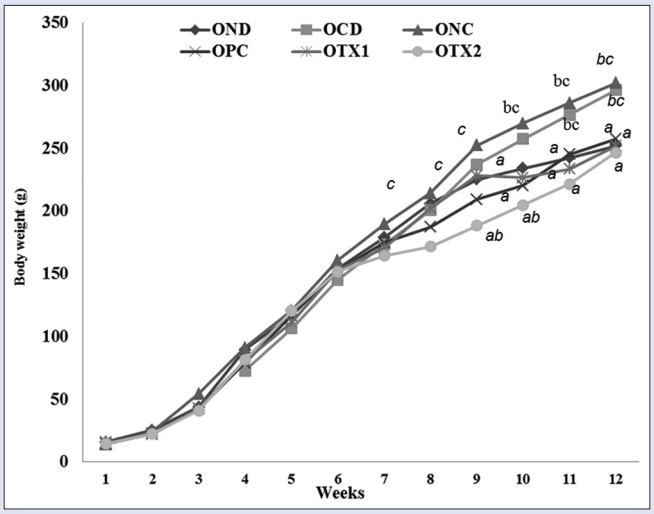

Figure 1: The effects of *Elateriospermum tapos* on total body weight of adult female offspring rats (n = 6) from 1 until 12 weeks of age. O: Indicates offspring; ND: Normal Diet; CD: Cafeteria Diet; NC: Negative Control (HFD); PC: Positive Control (200 mg/kg Orlistat); TX1: Treatment 1 (200 mg/kg *Elateriospermum tapos* seed) and TX2: Treatment 2 (200 mg/kg *Elateriospermum tapos* shell). Data are expressed as mean ± standard error mean and were analyzed by one-way ANOVA, followed by *post hoc* least significant difference. Significant level set at $P < 0.05$. Significant level set at $P < 0.05$. a: $P < 0.05$ versus NC, b: $P < 0.05$ versus ND, c: $P < 0.05$ versus PC

higher of total adipose tissues than that fed with normal diet (chow) by 168% and 131%, respectively. However, offspring from dams treated with *E. tapos* (OTX1 and OTX2) both showed slightly decreased in total adipose tissues, but it is not significant ($P > 0.05$) when compared to ONC. The similar result was observed for RpWAT [Table 1].

Effect of *Elateriospermum tapos* on kidney and liver profile test of adult female offspring

The liver function test showed ALT concentrations in HFD treated (OTX2) only was significantly ($P < 0.05$) lower compared to HFD fed group (ONC). Meanwhile, for kidney profile test, creatinine concentrations of offspring from HFD group (ONC) was significantly ($P < 0.05$) 33.96% increase compared to normal diet group (OND). The level of creatinine was significantly lower ($P < 0.05$) for all HFD-treated groups (OPC, OTX1, and OTX2) relative to the HFD group (ONC) by 24.13%, 27.94%, and 30.8%, respectively [Table 2].

Effects of *Elateriospermum tapos* on liver, heart, kidney, and retroperitoneal white adipose tissue histopathology of female offspring

Based on Figure 3, the liver histology of HFD and cafeteria groups (OCD and ONC) had presence of numerous ballooned cells; however, no steatosis and lobular inflammation was observed (score 2). Liver section of *E. tapos*-treated groups (OTX1 and OTX2) showing normal strands of hepatocytes in a single plate, normal sinusoids, and central vein (score 0). However, histological examination on heart of all groups showed no significant changes. The heart muscle was striated, like skeletal muscle with single central nucleus and the cells are branched thus highly connected by specialized junction called intercalated discs. Similarly, for kidney, there are none of glomerular histological changes were recognized [Figure 3].

HFD intake also led to marked adipocyte enlargement in HFD and cafeterias, OCD and ONC groups. As shown in Figure 3, more fat accumulation and subsequently marked extension of the size of adipocytes were seen. Lower adipocytes and more fat deposits could be found in HFD-fed groups as opposed to the normal diet control group. Apparently, the prepregnancy supplementation of *E. tapos* shell among dams has reduced the size of adipocytes in adult female children (OTX2), similar to Orlistat (OPC), considerably.

Effect of *Elateriospermum tapos* on the deposition of atherosclerotic lesion in adult female offspring

In the FND group which was on normal diet, no atheromatous lesion was observed. Whereas, the atherosclerotic lesion (black arrow) was observed in ONC, OCD, and OPC groups due to cumulative exposure of the aortic walls to cholesterol. As a result of *E. tapos* seed and shell supplementation in dams, OTX1 and OTX2 showed no present of atheromatous lesion and it was comparable with OND group [Figure 4].

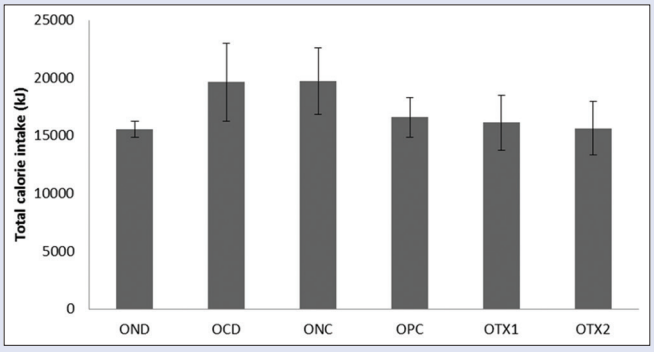

Figure 2: The effects of *Elateriospermum tapos* on total calorie intake of adult female offspring rats ($n = 6$) at 12 weeks of age. O: Indicates offspring; ND: Normal Diet; CD: Cafeteria Diet; NC: Negative Control (HFD); PC: Positive control (200 mg/kg Orlistat) TX1: Treatment 1 (200 mg/kg *Elateriospermum tapos* seed) and TX2: Treatment 2 (200 mg/kg *Elateriospermum tapos* shell). Data are expressed as mean ± standard error mean and were analyzed by one-way ANOVA, followed by *post hoc* least significant difference. Significant level set at $P < 0.05$. Significant level set at $P < 0.05$. $^{a}P < 0.05$ versus NC, $^{b}P < 0.05$ versus ND, $^{c}P < 0.05$ versus PC

DISCUSSION

Reportedly, HFCD causes increased in total body fat and fat distribution in obese animal models.[14] Study by Kokila *et al.*[10] proved that obese rats consumed HFCD, shows increase of body weight and calorie intake than lean rats consumed normal diet. *E. tapos* seed and shell prior to pregnancy of obese dams showed slight reduction in dams' body weight compared with HFCD dams group and the effect was remains in their PND21 offspring.[12] Our study into the impact of *E. tapos* seed and shell on adult female offspring revealed similar results in which adult female offspring from obese HFCD dams had increased in body weight compared to offspring of rats fed with normal diet. While, supplementation of either *E. tapos* seed or shell prior pregnancy of HFCD dams producing adult offspring with the body weight comparable with offspring from normal diet group.

Based on previous research, *E. tapos* have rich sources of polyunsaturated fatty acids and it consists of high percentage of oleic acid, linoleic acid, flavonoid acid, and alpha-linolenic acid (ALA).[8] The major *E. tapos* components have been found to be effective in weight gain and obesity prevention, respectively. This plant's active components can be related to the potent effect of *E. tapos* in HFCD feed both obese dams rats and their offspring. Numerous studies have shown that maternal obesity during pregnancy has an adverse impact on children's health in the short

Table 1: Organ and tissue weight of adult female offspring at 12 weeks of age

	Dietary group					
	OND (n=6)	OCD (n=6)	ONC (n=6)	OPC (n=6)	OTX1 (n=6)	OTX2 (n=6)
Liver (g)	7.97±0.47	9.31±0.76	9.69±0.89	9.74±0.36	8.14±1.19	8.27±0.68
Kidney (g)	2.24±0.27[a]	2.04±0.24	1.75±0.08[b]	1.80±0.13	1.80±0.12	1.68±0.06
Heart (g)	1.04±0.04	0.99±0.18	0.94±0.02	0.99±0.10	0.95±0.06	0.84±0.06
Total adipose tissue (g)	5.29±0.44	12.24±1.04	14.20±2.79	11.58±1.96	13.80±1.95	10.24±1.18
RpWAT (g)	2.55±0.3	5.95±1.13	6.01±0.78	5.40±0.77	5.44±1.99	4.53±0.90

Significant level set at $P<0.05$. $^{a}P<0.05$ versus ONC, $^{b}P<0.05$ versus OND, $^{c}P<0.05$ versus OPC. Data are expressed as mean±SEM and were analyzed by one-way ANOVA, followed by *post-hoc* LSD. Letter (O) indicates offspring. ND: Normal diet; NC: Negative control (HFD); PC: Positive control (200 mg/kg Orlistat); TX1: Treatment 1 (200mg/kg *E. tapos* seed); TX2: Treatment 2 (200 mg/kg *E. tapos* shell); *E. tapos*: *Elateriospermum tapos*; HFD: High fat diet; SEM: Standard error of mean; ONC: Offspring negative control; OND: Offspring normal diet; RpWAT: Retroperitoneal white adipose tissue; OPC: Offspring positive control

Table 2: Effect of *Elateriospermum tapos* on kidney and liver function test of female offspring at 12 weeks of age

	Dietary group					OTX2 (*n*=6)
	OND (*n*=6)	OCD (*n*=6)	ONC (*n*=6)	OPC (*n*=6)	OTX1 (*n*=6)	
Liver profile (mmol/L)						
AST (mmol/L)	126.17±23.70	168.67±21.43	171.50±31.01	150.83±17.47	142.17±17.92	142.00±13.67
ALT (mmol/L)	31.67±4.05[a]	41.00±3.97	48.33±5.86[b]	39.33±3.27	38.17±5.09	32.67±0.84[a]
Kidney profile (mmol/L)						
Creatinine (mmol/L)	34.67±0.80[a]	41.17±4.56[a]	52.50±4.42[b,c]	39.83±5.11[a]	37.83±1.60[a]	36.33±1.36[a]

Significant level set at $P<0.05$. [a]$P<0.05$ versus ONC, [b]$P<0.05$ versus OND, [c]$P<0.05$ versus OPC). After treatment, data are expressed as mean±SEM and were analyzed by one-way ANOVA, followed by *post hoc* LSD. Letter (O) indicates offspring. ND: Normal diet; NC: Negative control (HFD); PC: Positive control (200 mg/kg Orlistat); TX1: Treatment 1 (200 mg/kg *E. tapos* seed) and TX2: Treatment 2 (200 mg/kg *E. tapos* shell); *E. tapos*: *Elateriospermum tapos*; HFD: High fat diet; SEM: Standard error of mean; ONC: Offspring negative control; OND: Offspring normal diet; RpWAT: Retroperitoneal white adipose tissue; OPC: Offspring positive control; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase

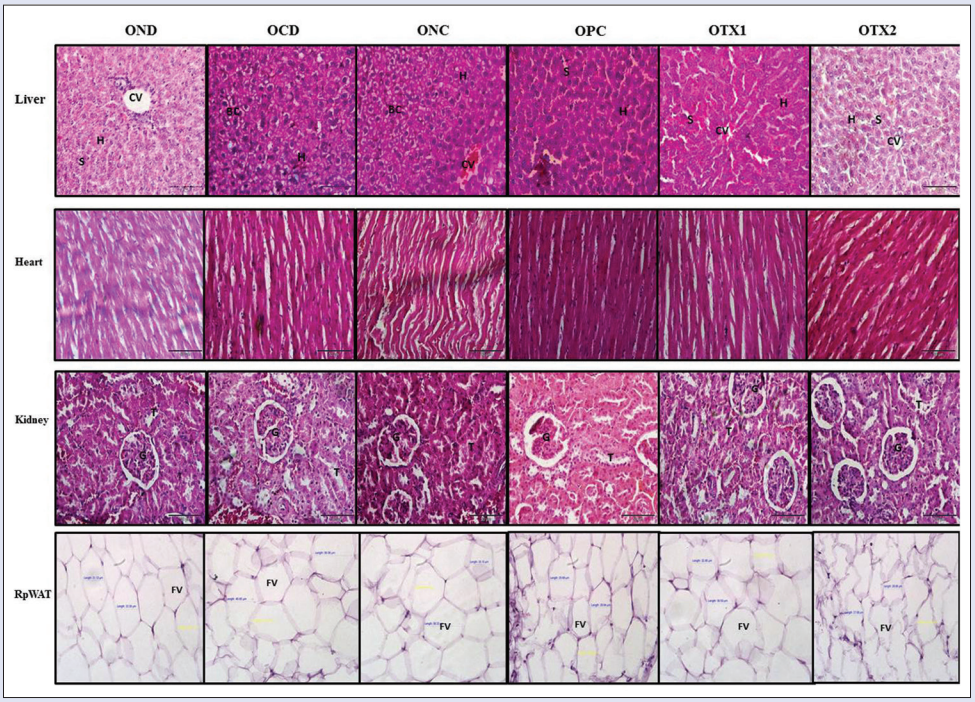

Figure 3: Photomicrographs of female offspring rat liver, kidney, heart and RpWAT sections stained with hematoxylin and eosin (×40). O: Indicates offspring; ND: Normal Diet; CD: Cafeteria Diet; NC: Negative Control (HFD); PC: Positive Control (200 mg/kg Orlistat); TX1: Treatment 1 (200 mg/kg *Elateriospermum tapos* seed) and TX2: Treatment 2 (200 mg/kg *Elateriospermum tapos* shell). CV: Central Vein; H: Hepatocyte; S: Sinusoid; BC: Ballooning cell; FV: Fat vacuole; N: Nucleus; G: Glomerulus: Magnification bar: 50 µm

and long term, such as increased risk of developing obesity and chronic diseases such as type 2 diabetes, which can lead to premature mortality in adult offspring.[15] In the current study, we observed the beneficial effects of *E. tapos* supplementation on the body weight in female adult offspring, normalizing the increase in maternal obesity-induced body weight, thus preventing the development of chronic diseases among the adult offspring.

Total adipose tissues showed that the offspring rats fed cafeteria diet (OCD and ONC) for 12 weeks had significantly higher of total adipose tissues and RpWAT than that fed with normal diet. Meanwhile, supplementation of *E. tapos* to obese dams showed slightly decreased in total adipose tissues and RpWAT in their adult female offspring. *E. tapos* shell group (OTX2) showed stronger impact relative to the seed group of *E. tapos* seed group (OTX1) although not significantly different. *E. tapos*, particularly in its shell, consists of higher flavonoid acid. Flavonoid acid promotes oxidation in adipocytes. Flavonoid acid also helps to regulate adipose deposition through numerous cell-signalling pathways.[16] Hence, the higher intake of flavonoid acid causes the reduction in fat mass which then reduces the body weight. Results revealed that *E. tapos* seed and shell induced total body weight reduction in adult offspring without affecting much on the total adipose tissue weight loss and calorie intake. Slightly decreased in calorie intake of groups treated with *E. tapos* possibly due to the feelings of fullness and decreases the hunger which induced by ALA that helps in enhancing the satiety feelings and decreasing the food intake. Supplementation of *E. tapos* causes ALA contents in *E. tapos* alleviates the suppression of hypothalamic AMP-activated protein kinase (AMPK). In addition, ALA promotes genes expression that is involved in the appetite stimulation, for example, the leptin hormone. Furthermore, because of high contents of protein and fiber metabolism in *E. tapos*, it suppresses the appetite and changes some level in regulation of weight hormone.[17]

The liver function tests including aspartate aminotransferase (AST) and ALT and kidney function test, the creatinine was conducted to investigate if any toxic impacts arising from *E. tapos* supplementation. This evaluation is crucial in determining the safety of *E. tapos* for further applications. Previous study indicating that offspring at

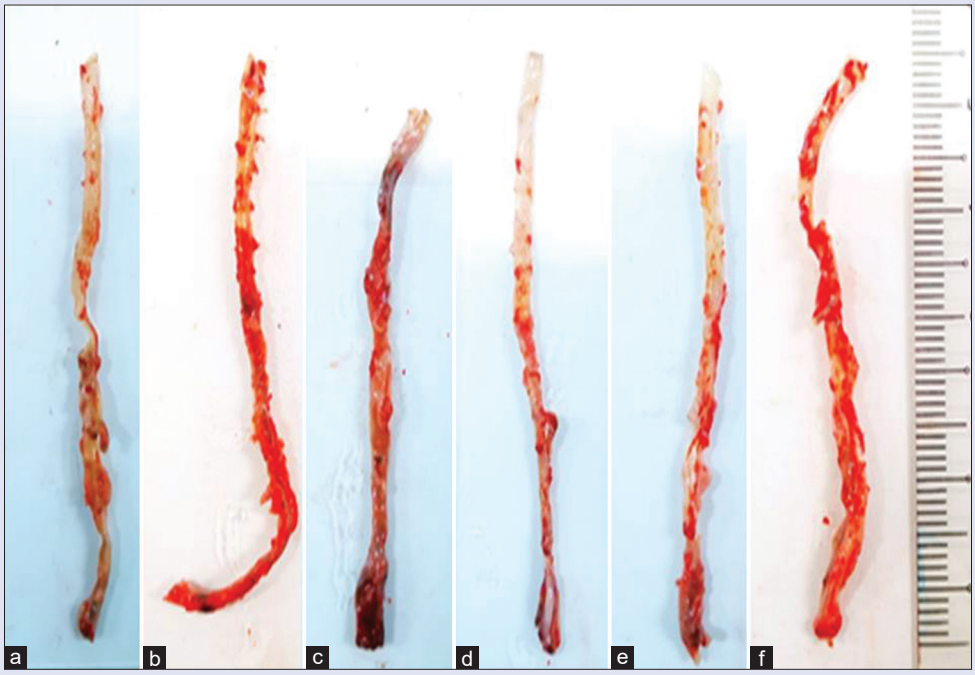

Figure 4: Effect of *Elateriospermum tapos* on the arterial tree of 12 weeks age of female offspring. O: Indicates offspring. ND: Normal diet; CD: Cafeteria diet; NC: Negative control (HFD); PC: Positive control (200 mg/kg Orlistat); TX1: Treatment 1 (200 mg/kg *Elateriospermum tapos* seed) and TX2: Treatment 2 (200 mg/kg *Elateriospermum tapos* shell). An arterial tree stained with Sudan IV of the atherosclerotic lesion in black color (black arrow). a;OND, b;OCD, c;ONC, d;OPC, e;OTX1, f;OTX2

PND21 from HFCD dams had high concentration of AST, ALT and creatinine while *E. tapos* shell supplemented dams producing PND21 with low AST, ALT and creatinine concentration.[12] However, our finding for liver function test showed only ALT concentrations in HFD treated (OTX2) lower compared to HFD fed group (ONC). Creatinine is one of the chemical waste products carried via the bloodstream and eliminated by the kidneys. Creatinine blood levels rise if the filtration in the kidney is deficient. After 12 weeks of examination, the creatinine level in 12th weeks of adult female offspring from HFD group (ONC) was increased compared to normal diet group (OND). Interestingly, the creatinine level for all HFD-treated groups (OPC, OTX1, and OTX2) were lowered as compared to HFD group (ONC). This finding demonstrated that administering *E. tapos* seed and shell in the normal diet (ND) and HFD-treated group (OTX1 and OTX2) was not complicated with any detectable adverse toxic effects in the studied rat.

Liver is the essential organ involved in compound detoxification and overall metabolism; thus, tests on liver function and histology are critical markers for disclosing the functional state. Previous studies found that hepatic steatosis, such as fat accumulation in hepatocytes during diet induced obesity, was characteristic of the liver in obese rats.[18] This result was the same with our finding, whereby the liver histology of HFD and cafeteria groups (OCD and ONC) had presence of numerous ballooned cells, however no steatosis and lobular inflammation. Differently, Santhra *et al.*[12] showed that PND21 offspring from HFCD dams only had few ballooned cells with no steatosis and lobular inflammation (score 1). Liver section of *E. tapos*-treated groups (OTX1 and OTX2) showing normal strands of hepatocytes in a single plate, normal sinusoids and central vein (score 0). *E. tapos* seed and shell supplementation in dams has effectively ameliorate the HFD-induced effect in liver cell among their adult offspring (OTX1 and OTX2).

Previous histological studies on the prolonged effect of HFD in rats reported the development of mesangial expansion in the kidney.[19] However, in all groups of our research, glomerular histological changes were not recognized. This result was comparable to research into the effect of obesity-induced glomerular inflammation, oxidative stress, and albuminuria in obese rats on Zucker male rats. Their finding has shown that obesity induction of only 8 weeks may trigger albuminuria, due to increased inflammation or oxidative stress, but may not be long enough to trigger pathological changes in the renal.[20] As our observation duration was only 12 weeks, although previous studies showing HFD-induced histological renal injuries were >3 months indicating that no renal modifications were present in our study.

Higher adipocytes and more fat deposits could be found in HFD-fed groups as opposed to the normal diet control group. However, the pre-pregnancy supplementation of *E. tapos* shell among dams has reduced the size of adipocytes in adult female children (OTX2), similar to Orlistat (OPC), considerably. ALA content in *E. tapos* alleviates the suppression of hypothalamic AMPK. In addition, ALA promotes genes expression that is involved in the appetite stimulation, for example, the leptin hormone. Leptin is a hormone that is secreted by adipocyte and in the presence of insulin, leptin inhibits AMPK in hypothalamus, thus, prevents overnutrition and suppressing appetite. The expression of leptin hormone plays important roles in the satiety and antiobesity.[21] According to study by Lecoutre *et al.*[22] elevatedleptin hormone causes rapid gain weight in offspring of HFD induced obese dams during the lactation. Therefore, regulation of leptin hormone and AMPK activity is important because it helps in decreasing food intake on offspring of obese dams.

In addition, AMPK activation reduced fat accumulation by inhibition of PPARγ and adipogenesis. Adipogenesis is formation of fat cell in which preadipocytes developed into mature adipocytes. This method will store energy as fat and obese the subjects.[23] Many obese models observe

a reduction in adipose tissue due to AMPK activation.[24] Previous study also stated that fatty acid like ALA prevents the development of HFD-induced adiposity and adipocyte hypertrophy in rats.[25] Moreover, ALA give effect on body fat by promoting the gene expression that regulates the adipose tissue metabolism. The gene expressions of ALA increase fat oxidation and decrease the fat storage.[26] In present study, the reduction inrpWAT weight was shown among offspring of obese dams supplemented with *E. tapos* seed and shell (OTX1 and OTX2) compared to HFD, ONC group. Histopathological examination of rpWAT was conducted in this research to confirm the antiadipogenic of *E. tapos*. Results in this research have shown that *E. tapos*' antiobesity activity is apparent from the decreased adipocyte size in *E. tapos*-treated groups. In contrary, adipocyte hypertrophy was increased in ONC and the total amount of adipocyte cell was decreased as compared to the OND group. It has been suggested that hypertrophy of adipocyte may occur to achieve the requirements for extra fat storage capacity in the development of obesity. However, further studies need to be done to identify how HFD and genetics specifically influence hypertrophy and the total number of adipocyte cell.[27]

CONCLUSION

Based on physiological, biochemical, and histological analyzes, we have demonstrated that hot aqueous seed and shell extraction of *E. tapos* has effective antiobesity activities to minimize maternal obesity in female rat descendants. ALA in *E. tapos* helps decrease the calorie intake of obese dams as well as their first generation and reduces fat accumulation by suppressing the activation of AMPK activity in the hypothalamus, thus causes the reduction in body weight. Based on the outcomes of parameters of liver and kidney function and histopathological research, we discovered that *E. tapos* extraction seed and shell showed no side impacts in rat liver, heart, kidney, and adipose tissues. This research determined the clinical usefulness of *E. tapos* as a therapeutic agent in organ dysfunction induced by obesity and may assist to decrease the commercial antiobesity drug's side effects.

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Conflicts of interest

There are no conflicts of interest.

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