Effect of *Elateriospermum tapos* Extract as Coadjuvant in Ameliorating Maternal Obesity on Female Offspring at Weaning

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Abstract

This study is aimed to investigate the effect of *Elateriospermum tapos* supplementation prior to pregnancy stage among obese rats and its association with their offspring. Thirty adult female Sprague Dawley rats were assigned to 2 groups, 6 rats were assigned to normal control group (DCG) which were fed with standard chow and the remaining rats were assigned to high-fat diet group (HFD) and cafeteria food to generate obesity for a duration of 5 weeks. These obese rats were further divided into 4 groups for treatment phase. Negative control (DNG) rats were administered with normal saline, positive control (DPG) with 200mg/kg of orlistat drug, treatment 1 (DTX1) with 200 mg/kg of *E.tapos* seed and treatment 2 (DTX2) with 200 mg/kg of *E.tapos* shell daily for 6 weeks. Two female rats were placed in cage with one male rats for 24 hours mating. After confirmation of mating, all dams were designated as day 0 for gestation stage. There was a significant decreased on total cholesterol among DTX2 treated dams and their offspring also were observed. Histopathological grading of the liver section of DNG, ONG and offspring from positive (OPG) displayed changes with score 1 with the presence of ballooning hepatocytes. Histology of RpWAT of DTX1, DTX2, OTX1 and OTX2 showed normal adipocytes with similar pattern of DCG and offspring from control OCG group. In conclusion, *E.tapos* shell had a greater effect on ameliorating maternal obesity on female offspring at postnatal day 21.

Keywords: *Elateriospermum tapos*, maternal obesity, lipid profiles, toxicity

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Introduction

Obesity is one of the biggest threats to human worldwide and it is associated with type II diabetes, chronic coronary heart disease and stroke. Epidemic obesity among adults has increased as consequence of sedentary life style and increased consumption of fast and processed food [1]. It is also proven that increasing obesity rats due to metabolic abnormality in utero during fetus stage [1]. Numerous epidemiological studies of mother-child and animal have been conducted to develop intervention that influence diet during pregnancy and lactation period for the log-term benefit of the offspring [2]. Studies have shown that an individual develops obesity from the early life starting from fetus to adult due to nutritional experiences in during early postnatal development [2].
Various epidemiological, clinical and experimental studies have demonstrated the relationship between the fetal nutrient environment, patterns of postnatal growth and adult adiposity [4]. Hales and Barker (2000) [5] highlighted the possible connection between fetus and adult stage on cardiovascular and metabolic disease. However, the complete mechanisms which involved in the developmental programming of obesity are yet to be revealed. Clinical and experimental test indicates that offspring that was exposed to elevated nutrient supply in uterus due to maternal overnutrition or maternal obesity are at higher risk of obesity throughout the life course [3].

Incresaed obesity awarness among community, increased the usage of natural product as anti-obesity treatment compare to modern medicine due to a side effect of modern medicine for long term usage. Nowadays, usage of plant as alternative medicine became famous due to less toxic and with few or no side effects. Natural product as the whole plan or part of plant play important role as new therapeutic agent. Malaysia is rich with natural products and one of the promising plants known as *Elateriospermum tapos* has the potential to be used as an anti obesity agent due to its high flavonoids and phenolic content with help in lipid peroxidation [7]. *E.tapos* also known as buah perah by the local people can be found in abundant at Jengka Forest Reserve, Peninsular Malaysia [7].

In this study, we investigate the impact of *E.tapos* seed and shell supplementation in offspring of obese rat model. The rats were given *E.tapos* seed and shell supplementation prior to pregnancy stage. Following that, the body weight, calorie intake, biochemical assay such as lipid profile, AST, ALT, creatinine and tissue histology such as liver, RpWAT, kidney and heart of mother and offspring were measured and analyzed. Data from this study can reveal the beneficial effect of *E.tapos* as anti-obesity supplement for pregnant women.

Materials and Methods

Collection and confirmation of plant species

The fresh *E.tapos* was brought from a local farm in Maran, Pahang. The plant was sent for confirmation at Herbarium Biodiversity Unit (UBD) in Institute of Bioscience, Universiti Putra Malaysia (voucher no, UPM SK 3154/17).

Extraction of E.tapos seed and shell

The extraction process began by placing the ground sample of *E.tapos* seed and shell weighing 50g into a different conical flask and mixed with 500 ml of distilled water. Mixture of *E.tapos* seed and shell was diluted in a different conical flask and wrapped with aluminum

foil prior to incubation in water bath at 70 °C for 24 hours. The aqueous extraction of *E.tapos* seed and shell were filtered using Whatman paper no 1 and transferred into 50ml Falcon tube. Sample were then freeze dried and grounded into powder form. Samples were stored 20°C until further use [8].

Preparation of High-fat diet (HFD)

The high fat diet (HFD) was prepared according to Levin and Dunn-Meynell (2000) [9].HFD ingredient were blended and prepared in the Memmert U universal heating oven at 60 ºC for 2 hours. This HFD consisted of 68 % standard rat pellet (Gordon Specialty Stockfeed, Malaysia), 6 % corn oil (Vecorn), 20 % milk powder (Dutch Lady), and 6% ghee (Crispo) which reflected 414 kcal/100g with 40% fats, 17% of proteins and 43% of carbohydrate.

Animal experimental study design

All procedures involving animal was conducted under the approval of the Animal Care and Ethics Committee of Management and Science University, AE-MSU-073. Thirty young adult female age 5-6 weeks Sprague Dawley rats, weighing between 150g to 200g were housed at 20 ± 2 °C and maintained on 12:12 h light/dark cycle. The rats were acclimatized for one week with free access to standard chow and water. Six rats were assigned to the normal control group (DCG) fed with standard chow (306.2 kcal/100 g with 48.8% carbohydrate, 21% protein, and 3% fat). The remaining rats were given HFD with selected cafeteria-food such including marble cake (440 kcal/100g), beef sausage (260 kcal/100g), and savory snacks (566 kcal/100g) to generate obesity for 5 weeks. After 5 weeks, obesity confirmation was done by comparing the body weight of the DCG group and HFD group with significant 15% difference of bodyweight [10]. Later these obese rats were divided into 4 group; negative control (DNG) rats administered with normal saline, positive control (DPG) with 200mg/kg of Orlistat drug, treatment 1 (DTX1) group with 200 mg/kg of *E.tapos* seed and treatment 2 (DTX2) with 200 mg/kg *E.tapos* shell administered via oral gavage daily for 6 weeks. Following 6 weeks of *E.tapos* treatment, 2 female rats each group were placed in a cage with one male rat for 24 hours for mating purpose. Vagina smear was performed the next morning to observe the presence of sperms as confirmation of mating and was designated as day 0 out of 21-15 day of gestation phase. Within 2 days of birth, the number of pups in one dams was adjusted to 8-12 pups. Pups were weighed every 2 days until weaning PND21. The body weight and calorie intake of all rats were recorded weekly for 17 weeks.

Post-mortem and tissue collection

The dams and offspring from each group were culled at postnatal day 21 after 12 hours fasting overnight. Adult rats and offspring were deeply anesthetized and blood rapidly collected through cardiac puncture and then the rats were culled by decapitation. Organs such as liver, retroperitoneal white adipose tissue (RpWAT), brown adipose tissue (BAT), total adipose tissue (consisted of inguinal fat and visceral fat), kidney and heart were dissected and weighed. All tissues were fixed in 10 % neutral buffered formalin (NBF) for further histological analysis.

Plasma biochemistry

Blood (5ml) was collected in heparin tubes and centrifuged to obtain the plasma. Plasma of lipid profile total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDH), high-density lipoprotein (HDL), level of creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using Alere Cholestech LDX® Analyzer.

Histology analysis

The liver, kidney, heart, and RpWAT were fixed in 10% NBF overnight. Prior to tissue processing with different concentration which were 100%, 95%, 80% and 70% concentration of alcohol and xylene before embedded with paraffin wax. Sectioning process was done to obtain a thin layer of paraffin ribbon with tissue thickness between 4 µm to 7µm followed by ribbon fishing using the slide. All slides were stained using Hematoxylin & Eosin (H&E) stain. Tissues were observed under the microscope and histological changes were recorded [11].

Sudan IV

Sudan IV mainly used for detection of atherosclerotic lesion in arterial tree as indicator of presence the high cholesterol level in blood. 10g of Sudan IV powder well mixed with 1 L 70% alcohol in Scott bottle and let the mixture overnight at 60 °C**.** The Sudan IV solution were filtered using Whatman paper no 1 and the filtered sample was transmitted into another Scott bottle. The arterial fixed with NBF were washed with running water for 1 hour before immersed in 70% alcohol for 30 minutes. The arterial tree were placed in the Sudan IV solution at room temperature overnight and next day the arterial tree was dipped into 70% alcohol for 2-3 minutes before placed into fresh 70% alcohol for 30 minutes. After well stained, the arterial tree was placed in running tap water for 1 hour before storing into NBF solution. The Sudan IV stained arterial tree was photographed to identifying the presence of erythematous plaque [12].

Statistical Analyses

Statistical analyses were performed using SPSS 25.0 Windows software (SPSS) and results were expressed as mean ± SEM. All data were analyzed for normality test. Body weight and calorie intake of dams before the treatment were analyzed by independent sample *t*-test. Body weight, organ weight, calorie intake, and plasma biochemistry data of dams after the treatment were analyzed by one-way ANOVA, followed by post hoc LSD. In all analyses, a probability of $p<0.05$ was considered to be statistically significant.

Results and Discussion

Results

Effects of HFD and calorie intake of dams before E.tapos treatment

Consumption of HFD with cafeteria diet has led to weight gain among the dams. These dams consumed more calories (kJ) and were significantly heavier (18%) than the standard chow-fed rats. Our data showed significant ($p<0.05$) increase in body weight among the obese group as compared to the control group as indicated Table I.

Table I: Effect of high-fat and cafeteria diet on body weight and calorie intake before *E.tapos* treatment

	Control rats (n = 6)	Obese rats (n = 24)
Bodyweight (g)	207.87 ± 6.53 [a]	253.39 ± 5.10 [b]
Calorie intake (kJ)	5705.77 ± 406.90 [a]	8401.14 ± 342.64 [b]

Data are expressed as mean ± SEM and were analyzed by independent sample *t*-test. Significant level set at $p<0.05$. Different superscript letters [a, b] denote significant differences at $p<0.05$.

Effects of E.tapos treatment on the dams body weight and calorie intake

Body weight among all HFD group showed significantly higher weight ($p<0.05$) compared to DCG. DPG group showed significantly ($p<0.05$) reduction in body weight (14 %) as compared to DNG group. Following *E.tapos* seed and shell treatment, rats from DTX1 and DTX2 groups showed slight decreased in body weight by 3.4% and 5.14%, respectively as compared to the DNG group (Table II). The calorie intake was significantly higher ($p<0.05$) in all HFD groups compared to DCG group.

Effects of E.tapos treatment on the organ weight

After the treatment phase, RpWAT weight in DPG and DTX1 groups showed significantly ($p<0.05$) decreased (58.76% and 41.4% respectively) compared to DNG group. Similar result was observed in total adipose weight in DPG and DTX2 groups' rats. However BAT, kidney, and liver weight showed no significant change in all HFD groups.

Effects of E.tapos treatment on liver and kidney profile

AST and ALT level in DNG group showed significant ($p<0.05$) increased compared to DCG. DPG, DTX1, and DTX2 showed similar level compared to DCG. Creatinine level in DNG shows significant increased ($p<0.05$) compared to DCG. However group DPG, DTX1 and DTX2 show similar level as DNG.

Effects of E.tapos treatment on lipid profile

There was a significant decreased ($p<0.05$) of TC level among all HFD group compared to DCG group (Table 2). DPG and DTX2 groups showed the lowest level of TC compared DNG group. TG, HDL and LDL showed no significant ($p>0.05$) changes in all HFD groups (Table II).

Table II: Effect of HFD on body weight, calorie intake, organ weight and biochemistry of dams after *E.tapos* treatment

	DCG n=6	DNG n=6	DPG n=6	DTX1 n=6	DTX2 n=6
BW after treatment (g)	272.51 ± 7.45 [a,c]	352.74 ± 13.90 [b,c]	309.41 ± 13.44 [a,b]	340.77 ± 17.89 [b]	334.60 ± 4.94 [b]
Caloric intake (kJ)	6167.88 ± 551.45 [a,c]	15053.21 ± 365.54 [b]	14297.64 ± 472.16 [b]	14143.19 ± 828.16 [b]	14021.58 ± 764.08 [b]
Organ weight (g)					
RpWAT	0.74 ± 0.40 [a,c]	15.51 ± 2.35 [b,c]	6.41 ± 2.03 [a,b]	13.53 ± 2.07 [b,c]	9.09 ± 0.87 [a,b]
BAT	0.27 ± 0.13 [a]	1.45 ± 0.62 [b]	0.55 ± 0.18	1.15 ± 0.65	0.58 ±0.08
Total adipose	1.20 ± 0.58 [a,c]	22.83 ± 5.44 [b,c]	11.14 ± 2.52 [a,b]	19.03 ± 4.40 [b]	11.63 ±1.40 [a,b]
Liver	6.10 ± 2.02 [a]	9.45 ± 0.51 [b]	8.57 ± 0.55	8.91 ± 1.15	8.55 ± 0.61
Kidney	1.39 ± 0.28	1.67 ± 0.14	1.69 ± 0.05	1.78 ± 0.23	1.58 ± 0.12
Heart	0.75 ± 0.16 [a,c]	1.07 ± 0.09 [b]	1.11 ± 0.06 [b]	1.18 ± 0.12 [b]	0.87 ± 0.05
Lipid profile (mmol/L)					
TC	0.80 ± 0.17 [a,c]	1.52 ± 0.19 [b]	1.28 ± 0.07 [b]	1.53 ± 0.13 [b]	1.47 ± 0.19 [b]
HDL	0.32 ± 0.09	0.50 ± 0.13	0.37 ± 0.03	0.48 ± 0.06	0.45 ± 0.06
LDL	0.45 ± 0.17	0.65 ± 0.17	0.55 ± 0.13	0.70 ± 0.10	0.60 ± 0.04
TG	0.50 ± 0.15	0.70 ± 0.13	0.57 ± 0.06	0.78 ± 0.19	0.88 ± 0.27
Liver profile (mmol/L)					
AST	114.5 ± 22.99 [a,c]	218.50 ± 10.18 [b]	184.50 ± 8.40 [b]	138.50 ± 10.07 [a,c]	138.67 ± 7.27 [a,c]
ALT	49.50 ± 11.39 [a]	70.67 ± 3.25 [b, c]	51.83 ± 4.47 [a]	51.17 ± 1.92 [a]	59.00 ± 2.42
Kidney profile (mmol/L)					
Creatinine	34.00 ± 7.25 [a,c]	46.17 ± 1.38 [b]	46.83 ± 3.40 [b]	43.00 ± 3.10	46.00 ± 2.67 [b]

Abbreviations: BW, body weight, RpWAT, retroperitoneal white adipose tissue; BAT, brown adipose tissue. Total adipose tissue represents the sum of RP, Visceral, and Uterine WAT mass; TC, plasma total cholesterol; HDL, plasma high-density lipoprotein; LDL. Low-density lipoprotein; TG, triglyceride; AST, Aspartate transaminase; ALT; alanine transaminase. The first letter indicates dams (D); the second letter indicates the groups. DCG; dams control group, DNG, dams negative control, DPG, dams positive (treatment with Orlistat drug) group, DTX1, dams treatment with seed group and DTX2, dams treatment with shell group. Data are expressed as mean± SEM and were analyzed by one way ANOVA, followed by post-hoc LSD.

Significant level set at $p<0.05$. $^{a}p < 0.05$ versus negative control, $^{b}p < 0.05$ versus normal control, $^{c}p < 0.05$ versus positive control.

Effects of E.tapos treatment on the histology section of dams

Histology section was performed on the liver, RpWAT, heart and kidney. The photomicrograph is shown in Figure I. Based on the liver histology, liver section of a DCG, DPG, DTX2 and DTX2 showing normal strands of hepatocytes (H), sinusoids (S) and central vein (CV). It showed 0% of biopsied hepatocytes affected. Liver grading score 0 grading due to no present of steatosis, no lobular inflammation and no present of hepatocyte ballooning. However, liver sections from DNG showed present of hepatocyte ballooning (BC) with a normal central vein. Liver grading score 1 with few ballooned cells present with no steatosis and lobular inflammation (Panel A). Based on Figure I, histological section of RpWAT from DCG, DNG, DTX1 and DTX2 rat showed normal adipocytes sizes range between 18 µm to 19 µm and the number of adipocytes cell range between 70-80 number of cell/per field comparing with histological section photomicrograph of RpWAT from DNG group. DPG group show hypertrophy of adipocytes, size range between 50 µm to 55µm and the number of adipocytes cell range between 20-25 number of cell/per field (Panel B). Morphological of heart cell (Panel C) and kidney cell (Panel D) show no changes among all the group.

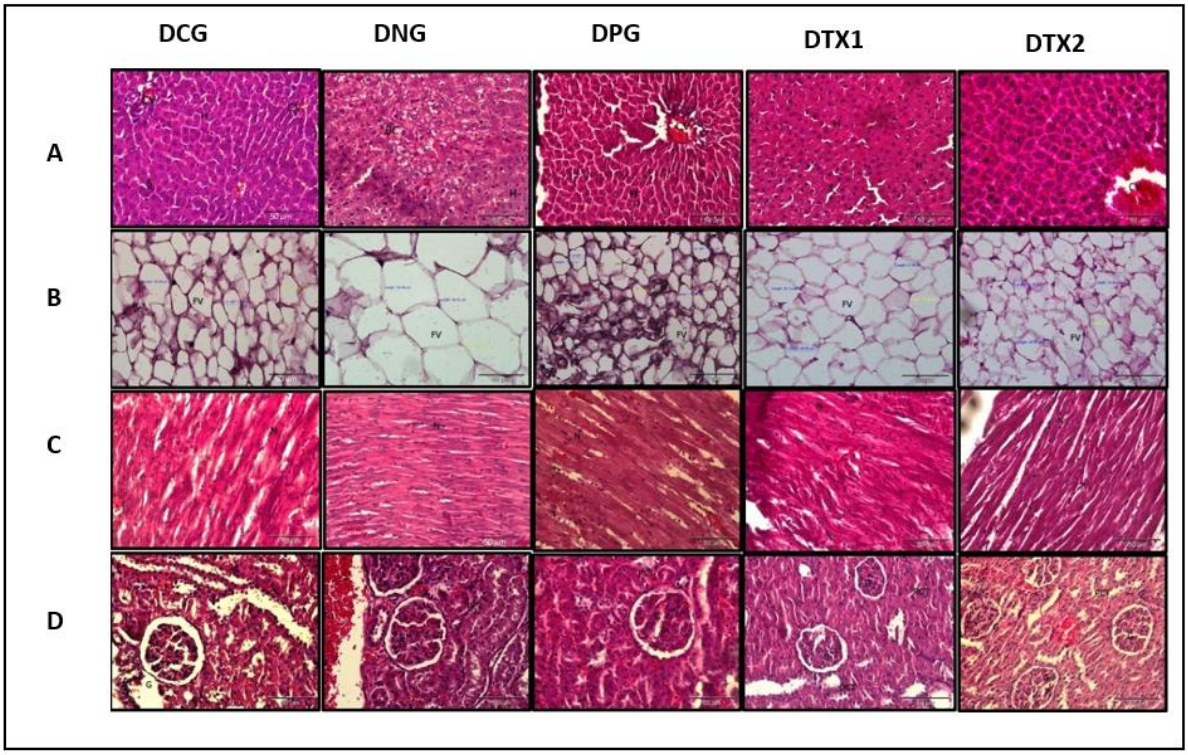

Figure I: Histology of liver, RpWAT, heart, and kidney of dams

Panel (A) hepatic histology, (B) RpWAT histology, (C) heart and (D) is renal. A (I) is liver histology from DCG, DNG, DPG, DTX1 and DTX2. B is RpWAT histology from DCG, DNG, DPG, DTX1 and DTX2. C is heart histology from DCG, DNG, DPG, DTX1 and DTX2. D is kidney histology from DCG, DNG, DPG, DTX1 and DTX2. CV, Central Vein; H, hepatocyte; S, sinusoid; BC, ballooning cell; FV, fat vacuole; N, nucleus; G, glomerulus: magnification bar: 50µm.

Effect of E.tapos on the deposition of atherosclerotic lesion in adult dams

An arterial tree which is stained with Sudan IV showed presence of the atherosclerotic lesion in black color. Group DCG showed no presence of the atherosclerotic lesion and similar pattern was observed in DPG, DTX1 and DTX2 group. However the DNG group showed clear presence of the atherosclerotic lesion through the arterial tree (Figure II).

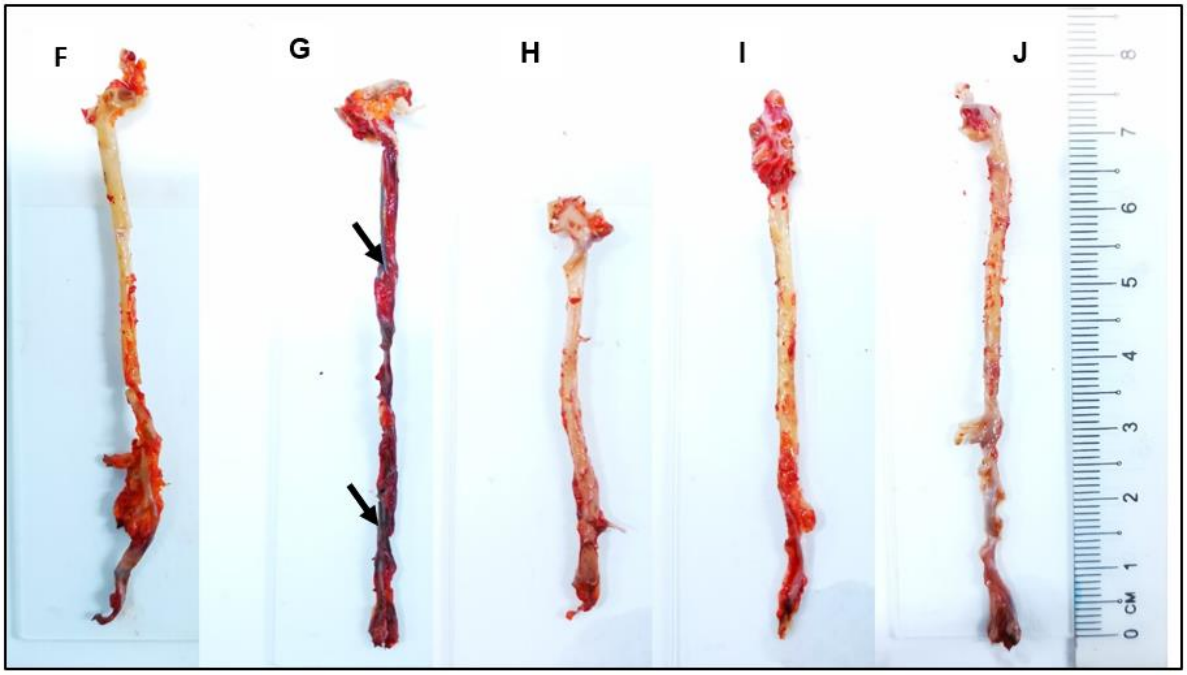

Figure II represent Sudan IV stain. Panel (F) Group DCG (dams control group), (G) Group DNG (dams negative group), (H) Group DPG (dams positive group), (I) Group DTX1 (dams treatment 1 group) and (J) Group DTX2 (dams treatment 2 groups).

The growth profile of female pups before weaning phase (PND1 to PND21)

The growth profile of the female pups from control and obese dams was observed by measuring the weight from Day 1 to Day 21. During weaning period, pups from obese dams were significantly ($p<0.05$) heavier that those from the normal chow group at PND21 (Figure III). Offspring from dams treatment group OPG, OTX1, and OTX2 showed similar body weight with the OCG. The body weight of all offspring groups was comparable until PND13 which the weight has become significantly heavier. At PND21, ONG group showed significant increased ($p<0.05$) in body weight as compared to the OCG group. Conversely, OTX1 and OTX2 groups showed significant ($p<0.05$) decreased in body weight when compared to ONG pups after lactation phase (Figure III).

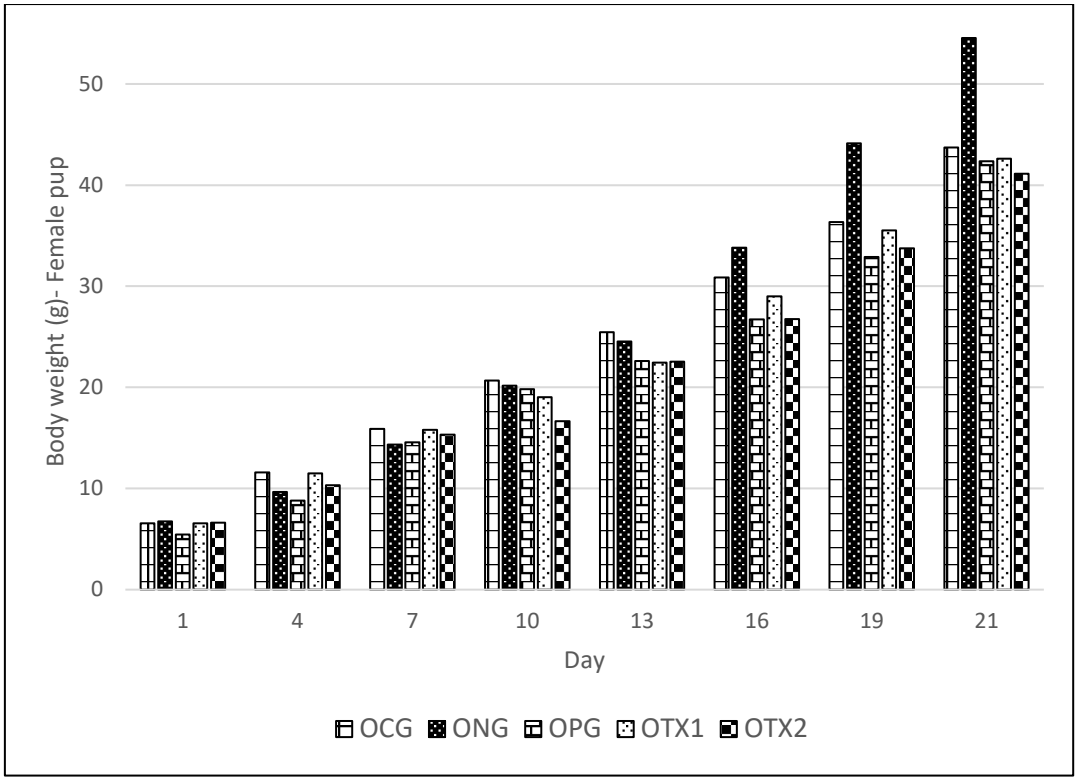

Figure III: Effect of *E. tapos* on body weight of female pups from treated dams from day 1 until day 21.
Abbreviations: the first letter indicate offspring (O); the second letter indicates the groups. OCG; offspring control group, ONG, offspring negative control, OPG, offspring positive group, OTX1, offspring from dams treat with seed group and OTX2, offspring from dams treat with shell group. Data are expressed as mean± SEM. Data are expressed as mean± SEM. Significant level set at $p<0.05$. $^{a}p < 0.05$ versus negative control, $^{b}p < 0.05$ versus normal control, $^{c}p < 0.05$ versus positive control.

Effect of E. tapos on the organ weight of female pups at PND21
In the female pups, total adiposity of OPG and OTX2 showed slight decreased (49.3% and 28.10%) compared to ONG group. RpWAT weight also show slight reduction in OPG, OTX1 and OTX2 compare ONG group (Table III). The liver weight of the obese and control offspring has not changed as seen in OCG and ONG groups. Kidney and heart weight show significant ($p<0.05$) changes among the offspring from HFD group compared to the offsping from control group (Table III).

Table III: Effect of *E. tapos* on organ weight of female pups at PND21

	OCG n=6	ONG n=6	OPG n=6	OTX1 n=6	OTX2 n=6
RpWAT	0.25 ± 0.17	0.42 ± 0.11	0.27 ± 0.07	0.29 ± 0.11	0.26 ± 0.10
BAT	0.15 ± 0.03 [a]	0.37 ± 0.12 [b]	0.18 ± 0.03	0.23 ± 0.05	0.27 ± 0.06
Total adipose tissue	0.50 ± 0.30	1.42 ± 0.53	0.64 ± 0.13	0.75 ± 0.17	0.72 ± 0.26
Liver	2.12 ± 0.32	2.71 ± 0.32 [c]	1.65 ± 0.15 [a]	1.87 ± 0.14	2.44 ± 0.52
Kidney	0.54 ± 0.06 [a]	0.91 ± 0.19 [b,c]	0.54 ± 0.05 [a]	0.56 ± 0.04 [a]	0.61 ± 0.12
Heart	0.26 ± 0.02 [a]	0.46 ± 0.11 [b,c]	0.22 ± 0.05 [a]	0.31 ± 0.04	0.24 ± 0.04 [a]

Abbreviations: BW, body weight, RpWAT, retroperitoneal white adipose tissue; BAT, brown adipose tissue. Total adipose tissue represents the sum of RP, Visceral, and Uterine WAT mass. The first letter indicates offspring (O); the second letter indicates the groups. OCG; offspring control group; ONG, offspring negative control; OPG, offspring positive group, OTX1, offspring treatment 1 group and OTX2, offspring treatment 2 group. Data are expressed as mean± SEM. Significant level set at $p<0.05$. [a]$p < 0.05$ versus negative control, [b]$p < 0.05$ versus normal control, [c]$p < 0.05$ versus positive control.

Effect of E. tapos on plasma biochemistry analysis of female pups at PND21

AST and ALT level in ONG group show higher level compared to other groups in female compared to another group. AST level of OTX1 and OTX2 in female offspring show significance changes ($p<0.05$) compare to the ONG group (Table IV). Creatinine level among control group offspring and obese group offspring showed no significant changes, however, the creatinine in ONG groups show higher level. There was a significant increase ($p<0.05$) in TC level among offspring pups from ONG, OPG, OTX1, and OTX2 compared to OCG group pups. However, the HDL, LDL and TG level among offspring from the obese group (ONG, OPG, OTX1, and OTX2) showed not significantly increased compared from pups from OCG group (Table IV).

Table IV: Effect of E. tapos biochemistry analysis in female pups at postnatal day 21

	OCG n=6	ONG n=6	OPG n=6	OTX1 n=6	OTX2 n=6
Lipid profile (mmol/L)					
TC	1.58 ± 0.07 [a, c]	2.13 ± 0.17 [b, c]	2.50 ± 0.14 [a, b]	2.50 ± 0.06 [a, b]	2.52 ± 0.10 [a, b]
HDL	0.43 ± 0.08	0.43 ± 0.08	0.62 ± 0.05	0.57 ± 0.03	0.62 ± 0.07
LDL	1.10 ± 0.28	0.77 ± 0.16	1.00 ± 0.37	0.83 ± 0.20	1.08 ± 0.17
TG	1.82 ± 0.77	2.95 ± 1.48	2.27 ± 0.73	2.57 ± 0.53	1.78 ± 0.46
Liver profile (mmol/L)					
AST	204.00 ± 3.98 [c]	230.67 ± 22.78	209.33 ± 12.13	152.17 ± 5.15 [b,a,c]	185.67 ± 14.53 [a]
ALT	43.33 ± 1.12	51.83 ± 1.47	45.67 ± 1.09	44.17 ± 4.74	42.67 ± 6.88
Kidney profile (mmol/L)					
Creatinine	21.67 ± 0.56	22.67 ± 0.96 [c]	19.17 ± 1.14 [a]	21.17 ± 0.40	19.83 ± 1.94

Abbreviations: TC, plasma total cholesterol; HDL, plasma high-density lipoprotein; LDL. Low-density lipoprotein; TG, triglyceride; AST, Aspartate transaminase; ALT; alanine transaminase. The first letter indicates offspring (O); the second letter indicates the groups. OCG; offspring control group; ONG, offspring negative control; OPG, offspring positive group, OTX1, offspring treatment 1 group and OTX2, offspring treatment 2 group. Data are expressed as mean± SEM. Significant level set at $p<0.05$. $^{a}p < 0.05$ versus negative control, $^{b}p < 0.05$ versus normal control, $^{c}p < 0.05$ versus positive control.

Effects of E.tapos treatment on the histology section of female offspring

Histology section was performed on the liver, RpWAT, heart and kidney of the female offspring and the photomicrograph is shown in Figure IV. Liver section (Panel K) of OCG, OTX1 and OTX2 showing normal strands of hepatocytes (H), sinusoids(S) and central vein (CV). It showed 0% of biopsied hepatocytes affected. Liver grading score 0 grading due to no present of steatosis, no lobular inflammation and no present of hepatocyte ballooning. However, liver sections of ONG and OPG showing present of hepatocyte ballooning (BC) with a normal central vein. Liver grading score 1 with few ballooned cells present with no steatosis and lobular inflammation (Figure IV). Based on Figure IV, histological section photomicrograph of RpWAT (Panel L) from OCG, OPG, OTX1 and OTX2 show normal adipocytes sizes range between 19 µm to 20 µm and the number of adipocytes cell range between 90-100 number of cell/per field comparing with histological section photomicrograph of RpWAT from ONG show hypertrophy of adipocytes, size range between 25 µm to 30 µm and the number of adipocytes cell range between 50-60 number of cell/per field.

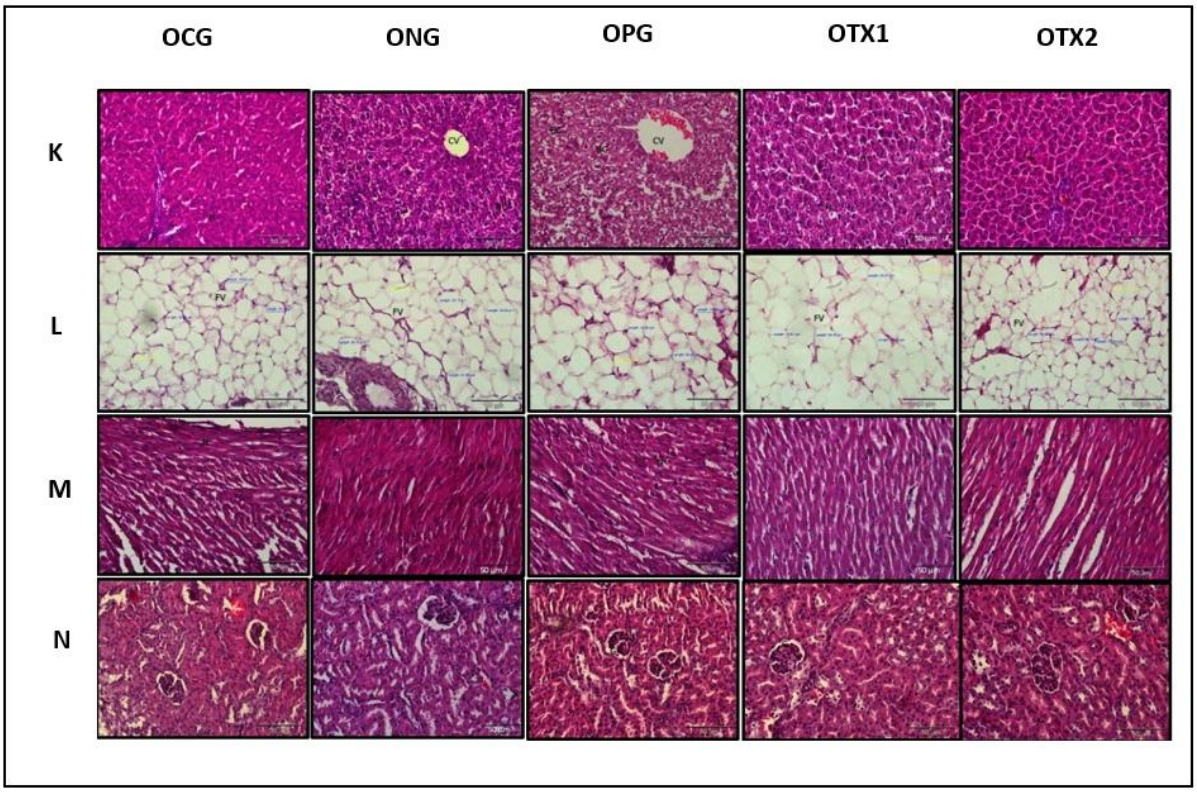

Figure IV: Histology of liver, RpWAT, heart, and kidney of female offspring PND21.
Panel (K) hepatic histology, (L) RpWAT histology, (M) heart and (N) is renal. K is liver histology from OCG, ONG, OPG, OTX1 and OTX2. L is RpWAT histology from OCG, ONG, OPG, OTX1 and OTX2. M is heart histology from OCG, ONG, OPG, OTX1 and OTX2. N is kidney histology from OCG, ONG, OPG, OTX1 and OTX2. CV, Central Vein; H, hepatocyte; S, sinusoid; BC, ballooning cell; FV, fat vacuole; N, nucleus; G, glomerulus: magnification bar: 50µm.

Effect of E.tapos on the deposition of atherosclerotic lesion in female offspring
An arterial tree which is stained with Sudan IV showed presence of the atherosclerotic lesion in black. Group OCG showed no presence of the atherosclerotic lesion and similar pattern was observed in ONG, DTX1 and DTX2 group. However, group DNG show only small lesion of atherosclerotic (Figure V).

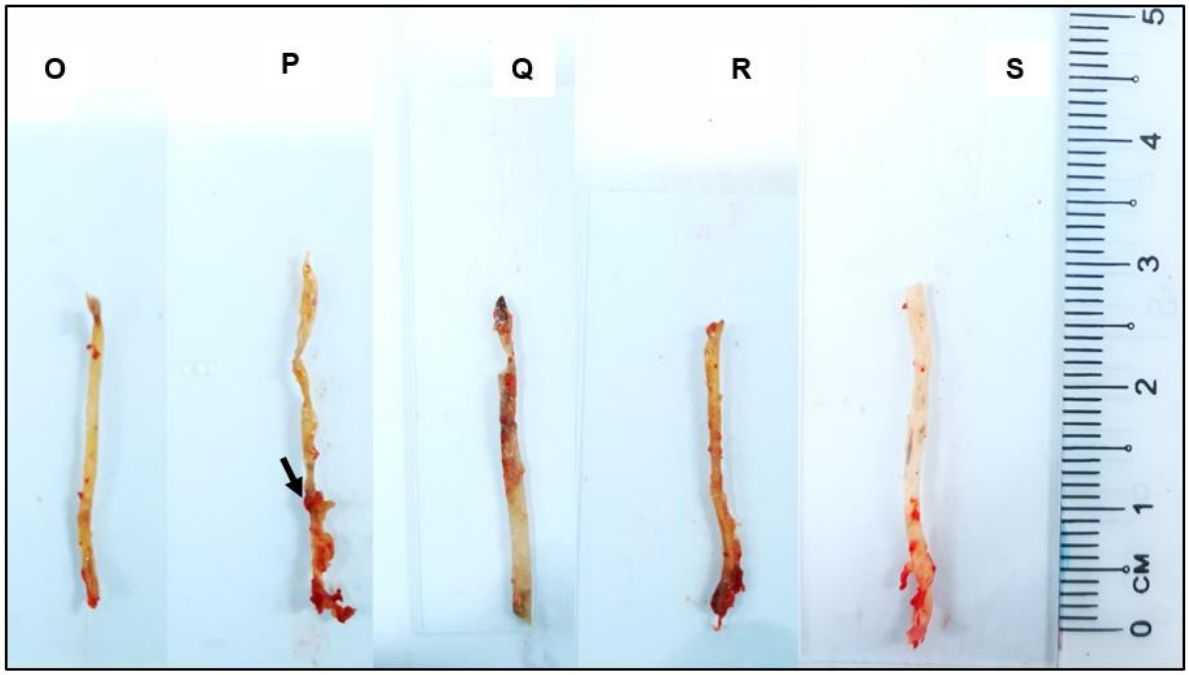

Figure V represent Sudan IV stain. Panel (O) Group OCG, (P) Group ONG, (Q) Group ONG, (R) Group OTX1 and (S) Group OTX2.

Discussion

Obesity is associated with high intake of food with less physical activity which will lead to dysfunctional appetite control [13]. Body mass and appetite during pregnancy (maternal obesity) can alter the appetite control and also body weight of offspring. Until recent, only two drugs, orlistat, and sibutramine available and approved for long term usage. All this medicine have known side effect during the curing process of obesity. Therefore the natural product is an alternative way to treat this obesity because it may cause less side effect [13]. There are some research have been conducted using the natural product as anti-obesity [14]. One of the recent local natural product that showed properties as anti-obesity is *Elateriospermum tapos* (*E.tapos*) which is known as buah perah by local people. The effect of *E.tapos* seed and shell were examined in few parameters such bodyweight of dams and offspring, calorie intake in dams, retroperitoneal adipose tissue weight, brown adipose tissue weight and biological assay on dams and offspring. In this study, the main purpose was to explore the effect of *E.tapos* during maternal obesity and its effect on offspring. There were several studies that have been conducted to determine the effect of maternal obesity on the offspring and many interventions have been done such as exercise during pregnancy particularly to discover the most efficacy treatment for obesity [15]. In this study, it was shown that HFD food that contain high fat level and cafeteria food with high level sugar had caused obesity among Sprague Dawley rats. According to Bahari (2013) [15], maternal obesity can activate hyperphagia and thus lead to an increased fat accumulation among offspring. The current research showed that the offspring from DNG which fed with was HFD and cafeteria diet for 5 weeks has led to increased the body weight and adiposity in offspring. Previous research show that, rat fed with high fat contain food and high sugar level will lead to palatable food and inhibiting the satiety signal and up regulates hunger sensation. This process will lead increased in body weight and calorie intake in human and also in animal. Researcher also conclude that there are positive relationship between the level of fat and sugar in the food will caused obesity [16]. The changes

in histopathological of RpWAT show that rat with higher body weight (DNG) show hypertrophy of adipocytes cell with range of 50µm to 55µm with less cell count under per field compared to less weight rats group (DCG, DPG, DTX1 and DTX2) rat showed normal size adipocytes which ranged from 18µm to 19µm and more cell count under per field.

A study conducted Nor Liyana et al (2019) [17, 18] has reported that *E.tapos* shell consist high content of flavonoids know as 3'4'5' Trimethoxyflavone compared to the *E.tapos* seed that make *E.tapos* as he potential anti-obesity agent. Flavonoids helps in reducing fat deposition by increasing beta-oxidation of fatty acids [18]. Flavonoids (3'4'5' Trimethoxyflavone) in *E.tapos* shell help inhibit the adipogenesis by reducing the transcriptional activity of PPARy and it enhances lipolysis [19, 20]. A study also reveal that *E.tapos* shell has high level of content lipase inhibitor, α-amylase, and α- glucosidase inhibitor which it able inhibiting the main digesting enzyme such as lipase and carbohydrate [17, 18].This will help in the absorption and digestion of nutrient, it leads to reducing calorie intake and controlling energy [18,19]. In contrast, the *E.tapos* seed have lower level of flavonoids compare to *E.tapos* shell which is giving less significance as anti-obesity agent. Thus, it reduce the body weight, calorie intake and the size of RpWAT in *E.tapos* shell treated group.

In this study, TC level and HDL level show decreasing among *E.tapos* supplemented dams and offspring and it proved that *E.tapos* help inhibit the cholesterol level and adipogenesis by reducing the transcriptional activity of PPARy by enhances lipolysis. Few research conducted show that flavonoids intake has been helping in reducing LDL level and HDL level among patient diagnosis with hypercholesterolemia [21, 22]. The present study show that *E.tapos* supplement particularly *E.tapos* shell which contain flavonoids help in fat oxidation by removed the accumulation of fat and decrease the fatty acid synthesis. This can support by the result of deposition of atherosclerotic lesion in DPG and ONG group compare to other group.

Nonalcoholic fatty liver disease (NAFLD) is very common among most of the obese people and it became a major concern nowadays. NAFLD leads to increasing level of hepatic triglyceride and it lead to hepatic steatosis [25]. Previous study has shown that intake of high calorie food such as high level of fat food and sugar can cause abnormality in mitochondria and mononuclear inflammation [25]. It can also cause lobular inflammation, fibrosis and hepatocellular ballooning due to mitochondrial destruction [25]. Previous study has proven that the main pathogenesis of NAFLD is due to abnormal secretion of adipocytokines including adiponenctin, leptin and resistin [25]. The H &E staining in recent study revealed that rats from HFD show the present of ballooning cell with score 1 compare to *E.tapos* treated group. This result showed that *E.tapos* supplemented has been help reducing the lipid deposition in liver compared to the HFD group rats. This result reveals that supplementation of *E.tapos* shell with high level of flavonoid has good potential on decreased hepatic lipid accumulation by and improve the liver function.

This impact of maternal obesity was passed onto the next generation of offspring. Offspring from obese dams show higher body weight compare to offspring from treatment group. Current study also showed that maternal obesity promoted post weaning effect on the offspring through adipogenesis (increased RpWAT) among offspring from HFD induced dams compared to offspring from treated group. Previous study also proven that rat which consume HFD significantly caused increased in body weight and calorie intake among offspring [26]. It proven that alteration in leptin and insulin level in dams fed with HFD during pregnancy and

lactation and this had change to offspring. Supplementation of *E.tapos* as show that it can ameliorating the maternal obesity effect to female offspring.

Conclusion

In this study, the effects *of E.tapos* seed and shell in ameliorating maternal obesity on female offspring was determined through assessment of body weight, calorie intake, adiposity weight, and biochemical assay. Data from this study highlights the potential use of *E.tapos* seed and shell for the treatment of obesity. This study proved that *E.tapos* shell more effective as anti-obesity supplement compared to *E.tapos* seed

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors report no conflicts of interest in this work.

Compliance with Ethical Standards

The work is compliant with animal ethical standards.

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