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Elateriospermum tapos Supplementation in Dams Ameliorating Obesity Development and Stress Hormone Level among Adult Male Offspring †

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Abstract: Maternal obesity is a metabolic disorder described by chronic inflammation, an increase of stress hormone and influences non-communicable diseases in offspring. *Elateriospermum tapos* has potential as an antioxidant and inhibitor of lipid (pancreatic lipase) and carbohydrate (α-amylase and α-glucosidase) which are beneficial to combat obesity and diabetes. This study was aimed to investigate the effect of *E.tapos* supplementation in obese rat prior pregnancy on the dams' and male offspring body weight, and the level of stress hormone; Adrenocorticotropic hormone (ACTH) and Corticosterone (CORT). Thirty female Sprague Dawley rats were used in this study. Six rats were assigned to a normal diet (DND) group fed with a standard chow diet. The remaining rats were fed with a high-fat and cafeteria diet (HFCD) to generate obesity for 5 weeks. The obese rats were further divided into 4 groups (n = 6/group); dams negative control group (DNC, normal saline), dams positive control group (DPC, 200 mg/kg of Orlistat), dams treatment 1 group (DTX1, 200 mg/kg of *E. tapos* seed) and dams treatment 2 group (DTX2, 200 mg/kg of *E. tapos* shell). The treatment was given for 6 weeks daily before mating. At weaning, male offspring were designated into 6 groups according to their dam's group (n = 6/group) and continue with cafeteria diet except for the control group. The offspring were culled at 12 weeks of age for blood sample collections. DTX2 and their male adult offspring showed significantly lower in body weight as compared to DNC and their male offspring. Male offspring from DTX2 also showed significantly low ACTH and CORT level as compared to male offspring from DNC group and comparable level with DND group. *E.tapos* shell supplementation was effective in reducing the development of obesity and suppresses stress through regulation of ACTH and CORT release in male adult offspring.

Keywords: maternal obesity; offspring; high-fat diet; cafeteria diet; stress hormone

1. Introduction

Obesity is an increasingly growing pandemic with significant public health implications. This pandemic initiated by a sedentary lifestyle and continuous availability of high caloric food option, with higher prevalence in women than in men, and its associated disorder such as cardiovascular disease, certain types of cancer and type 2 diabetes [1]. Evidence has been increasing in recent years that stress, particularly an increase in the glucocorticoid stress hormone cortisol, plays a role in the development of obesity [2]. A glucocorticoid secreted by the cortex of the adrenal gland is corticosterone. Corticosterone is formed by the adrenocorticotropic hormone (ACTH) in response to adrenal cortex stimulation and is the precursor of aldosterone. Cortisol is known to induce redistribution to the abdominal area of white adipose tissue and often increases appetite with a desire for energy-dense food ("comfort food") [3]. Abdominal obesity, metabolic syndrome (MetS), and ultimately cardiovascular diseases (CVD) arise in patients that are chronically exposed to elevated levels of glucocorticoids, such as in Cushing's syndrome or by using high doses of exogenous GC. Interestingly, the obesity pandemic in our modern society coincides with an increase in factors that stimulate the production of cortisol, such as chronic stress, high glycemic index food intake, and decreased sleep levels [2]. This demonstrates a vicious cycle in which increased glucocorticoid production, obesity and stress combine and exacerbate each other [4].

Increased use of traditional herbal medicine has contributed to the prevalent side effects of conventional obesity treatment, such as headache, constipation, heart arrhythmia and more diseases. As a result, procedures using natural ingredients with comparatively healthy and fewer side effects are gaining interest in curing obesity [5]. *Elateriospermum tapos (E.tapos)* is a tropical canopy found mostly in Southeast Asia, including Peninsular Thailand, Peninsular Malaysia, Sumatra, Java, and Borneo's tropical rainforests. White and sticky latex, traditionally used in the healing of wounds and broken soles in the treatment of feet [6,7] is present in *E.tapos* bark, leaves and fruit stalks. The predominant polyunsaturated fatty acid, *E.tapos* linolenic acid seed oil, has been of great benefit in the treatment of chronic diseases [6]. In 2019, Nor Liyana and colleague research [8] showed that *E.tapos* shell hot water extraction includes high phenolic and flavonoid, highlighting the potential of *E.tapos* as an anti-obesity agent [9]. The study has been extended and the anti-obesity effect of *E.tapos* shell extraction has been shown to decrease body weight and calorie intake in rats fed a high-fat diet [10]. Further research by Santhra et al. [11] on the effects of *E.tapos* seed and shell extraction on offspring at post-natal day 21 of obese dams showed that weight loss was promoted by the extraction of *E.tapos* in the dams and their offspring. The aim of this research was to investigate the impact of *E.tapos* in ameliorating obesity development and stress hormone level among adult male offspring.

2. Materials and Methods

2.1. Plant Material

E. tapos fruit was collected from Pahang, Malaysia and the Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia (voucher number SK3154/17) carried out the identification of the species. The fruit was dried overnight for 24 h in an oven with the temperature of 60 °C, followed by seed segregation from the shell, grounded and sieved. Fifty grams of mashed *E. tapos* were added to 500 mL of distilled water in a 1 L of Scott bottle wrapped in aluminium foil and held for 24 h in a water bath at 70 °C. The solution was let to cooled at room temperature (25 + 10 °C) followed by filtration process using Whatman paper No 1. Filtered solution was then concentrated using the freeze dryer (Scanvac). Until further use, the prepared sample was held at −20 °C [10–12].

2.2. Experimental Animal and Diet

All animal-related procedures were carried out under the approval of the involving animal were conducted under the approval of Management & Science University's Animal Care and Use Committee (AE-MSU-073). In this study, thirty female Sprague-Dawley rats weighing between 150 and 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 for 1 week in a temperature controlled room (21 ± 1 °C) on 12/12 h light/dark cycle. The rats were fed with standard chow diet and water was available ad libitum.

Six rats that were fed with regular chow were allocated to the normal diet group (Group 1, DND) and the rest of the rats were assigned to the high-fat diet group (HFD), which were fed with both high-fat diets and selected cafeteria foods like 440 kcal/100 g cake, 260 kcal/100 g sausage, and 566 kcal/100 g extruded savory snacks. In the cafeteria diet, nutritional products were chosen to reflect the variety, palatability and energy density of the modern western diet.

Obesity was verified after 5 weeks by comparing the significant 15 percent bodyweight difference between the DND and HFD groups [13]. The HFD groups were further divided into 4 groups according to their treatment; Group 2: DNC (Dams Negative Control, normal saline), Group 3: DPC (Dams Positive Control, 200 mg/kg Orlistat), Group 4: DTX1 (Dams Treatment 1, 200 mg/kg *E.tapos* seed supplementation) and Group 5: DTX2 (Dams Treatment 2, 200 mg/kg *E.tapos* shell supplementation). The treatment period was continued for 6 weeks. Calorie intake and bodyweight were measured weekly.

2.3. High Fat Diet

High fat diet was composed of 414 kcal/100 g, with 17% of protein, 40% fat, and 43% carbohydrate. All ingredients were blended and contained 6% corn oil (Vecorn), 6% ghee (Crispo), 20% milk powder (Dutch lady) and 68% standard chow pellet (Gordon Specialty Stock feed). Standard chow pellet contained 306.2 kcal/100 g with 21% protein, 3% fat, and 48.8% carbohydrate [10,11].

2.4. Mating, Gestation and Offspring

Female rats were mating with a normal diet fed to male rats after 6 weeks of treatment, and vaginal smears were conducted at 8 a.m. the next morning to search for sperm as evidence of successful mating, and this was designated as 0 day of gestation. Litter sizes were equalised to 8 to 12 pups per dam within 2 days of birth. The offspring were designated according to their dam's group; Group 1a (OND, offspring normal diet) and Group 1b (OCD, offspring cafeteria diet) both from Group 1 dams; Group 2a (ONC, offspring negative control) from; Group 3a (OPC, offspring positive control); Group 4a (OTX1, offspring treatment 1, *E.tapos* seed); Group 5a (OTX2, offspring treatment 2, *E.tapos* shell). After the weaning phase, offspring were fed with standard chow and cafeteria diet with the exception of Group 1a offspring that were fed with standard chow only.

2.5. Blood and Tissue Collection

Fasting blood samples were obtained from each animal at week 12 of age in the control and test group via cardiac puncture after each rat had been anesthetized. 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 until further analysis.

2.6. Determination of Corticosterone and ACTH in Plasma

Corticosterone and ACTH concentration of 12th weeks age of female offspring were determined with ELISA kit (Elabscience) utilized the principle of Competitive-ELISA. The micro ELISA plate provided in this kit has been pre-coated with Human Corticosterone or ACTH. During the reaction, rats Corticosterone or ACTH in the sample or standard competes with a fixed amount of Human Corticosterone or ACTH on the solid phase supporter for sites on the Biotinylated Detection Ab specific

to Human ACTH. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of rats corticosterone and ACTH in the samples is then determined by comparing the OD of the samples to the standard curve.

2.7. Statistical Analysis

Data were analyzed with IBM statistics 25.0 windows. Results were expressed as mean ± SEM. Data normality was evaluated using a normality test. Dams' body-weight, offspring bodyweight and level of Corticosterone and ACTH in Plasma were analyzed by one-way ANOVA followed by post hoc LSD. Probability of $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Effect of E. tapos on Dams Bodyweight (g)

Table 1 showed the effect of *E.tapos* on the total body weight of dams after lactation phase. Negative Control Dams (NCD) showed a significantly increased by 33.46% in body weight as compared to the normal diet dams (DND) group. There were no significant different for positive (DPC) and treatment 1 dams group (DTX1). However, treatment group 2 dams (DTX2) showed significantly ($p < 0.05$) decreased by 6.45% in body weight when compared to negative control (DNC) group.

Table 1. Effect of *E.tapos* on the total bodyweight of dams (n = 6).

	DND	**DNC**	**DPC**	**DTX1**	**DTX2**
Dams Bodyweight (g)	191.97 ± 38.72	275.42 ± 15.21 [b]	256.20 ± 18.41	291.91 ± 18.29 [a]	257.64 ± 5.69 [a]

Abbreviations: Letter (D) indicates Dams. ND: Normal Diet; NC: Negative Control; PC: Positive Control (Orlistat); TX1: Treatment 1 (*E.tapos* seed) and TX2: Treatment 2 (*E.tapos* shell). 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 DNC, [b] $p < 0.05$ versus DND, [c] $p < 0.05$ versus DPC. n = 6/group.

3.2. Effect of E.tapos Supplement on the Bodyweight of 4 to 12 Weeks Age Adult Offspring

Table 2 showed the total bodyweight of male offspring rat from 4 to 12 weeks of age. Result showed that bodyweight of male rats from the HFD group increased simultaneously as compared with the treatment groups. In male offspring, the cafeteria diet groups had 8.6% (OCD) and 17.4% (ONC) higher bodyweight as compared to the normal diet group (OND). Male offspring at 4, 6 and 7 weeks of age show a significant difference $p < 0.05$ in their bodyweight between all groups (Table 1). The bodyweight of OPC and OTX1 groups were significantly less $p < 0.05$ as compared to OND as early as at 4 weeks of age. While OTX2 group started to showed significantly $p < 0.05$ lower bodyweight at weeks 6 and 7 of age as compared to the ONC group.

Table 2. Effect of *E.tapos* on the total bodyweight of 4 to 12 weeks age male rats (n = 6).

	OND	OCD	ONC	OPC	OTX1	OTX2
Week 4	102.78 ± 9.73	91.90 ± 9.35	96.31 ± 5.67 [c]	78.13 ± 4.43	68.96 ± 4.93 [b]	85.94 ± 4.56
Week 5	131.36 ± 12.48	131.21 ± 9.06	134.27 ± 14.84	118.75 ± 6.82	105.89 ± 6.90	125.09 ± 7.27
Week 6	195.01 ± 12.36 [c]	169.79 ± 12.55	198.76 ± 8.63 [c]	160.50 ± 11.52 [ab]	154.50 ± 8.55 [ab]	164.36 ± 7.24 [ab]
Week 7	224.89 ± 16.13	224.59 ± 16.58	245.53 ± 13.15 [c]	188.68 ± 13.34 [a]	200.04 ± 11.53 [a]	191.79 ± 7.77 [a]
Week 8	262.00 ± 10.90	249.91 ± 11.75	259.85 ± 20.30	222.03 ± 6.41	211.99 ± 25.34 [ab]	233.75 ± 12.85
Week 9	288.02 ± 6.55	285.74 ± 10.48	311.27 ± 22.14	263.96 ± 9.24 [a]	261.87 ± 22.66 [a]	269.47 ± 12.42
Week 10	316.11 ± 5.36	312.75 ± 10.91	334.26 ± 22.14	305.58 ± 9.24	299.22 ± 22.66	305.93 ± 12.42
Week 11	328.32 ± 6.84	320.10 ± 14.18	351.12 ± 16.79	321.60 ± 12.40	305.88 ± 21.22 [a]	308.16 ± 6.30 [a]
Week 12	322.38 ± 15.11 [a]	350.32 ± 21.11	378.51 ± 11.08 [b]	351.98 ± 8.56	336.26 ± 21.99	330.95 ± 4.47 [a]

Abbreviations: Letter (O) indicates offspring. ND: Normal Diet; NC: Negative Control; PC: Positive Control; TX1: Treatment 1 (*E.tapos* seed) and TX2: Treatment 2 (*E.tapos* shell). 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 FNC, [b] $p < 0.05$ versus FND, [c] $p < 0.05$ versus FPC. n = 6/group.

3.2. Effect of E.tapos Supplement on the Corticosterone and ACTH Plasma Level of 12 Weeks Age Adult Offspring

Figure 1 showed the effect of *E.tapos* supplementation on the plasma level of corticosterone and ACTH of male adult offspring. Result showed that male adult offspring from HFD and cafeteria diet rats (ONC and OCD) had significantly ($p < 0.05$) increased in level of ACTH and corticosterone as compared to normal diet (OND) group. For ONC group the ACTH was two folds increased, while the corticosterone was one fold increased as compared to the OND group. Interestingly, the plasma ACTH and corticosterone level for offspring from dams supplemented with *E.tapos* seed and shell (OTX1 and OTX2) showed significant $p < 0.05$ decreased when compared with the offspring from HFD groups (ONC).

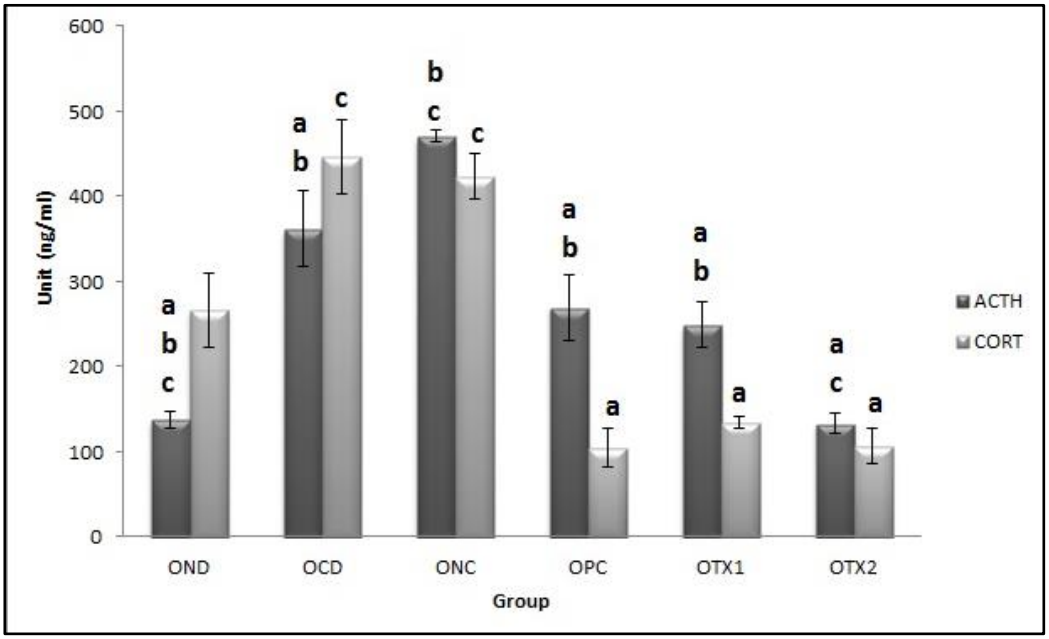

Figure 1. Effect of *E.tapos* supplement on the corticosterone and ACTH plasma level of 12 weeks age adult offspring. Abbreviations: Letter (O) indicates offspring. ND: Normal Diet; NC: Negative Control; PC: Positive Control; TX1: Treatment 1 (*E.tapos* seed) and TX2: Treatment 2 (*E.tapos* shell). Data 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 FNC, b $p < 0.05$ versus FND, c $p < 0.05$ versus FPC. n = 6/group.

4. Discussion

For proper development during childhood and adulthood, nutrition given during the prenatal process is important. In adulthood, offspring fed a high-fat diet are at high risk of obesity and metabolic disorders. In our study, we clarify the potential of *E.tapos* to improve the effect of maternal obesity on dams and the body weight of their male offspring and the plasma level of stress hormone which are ACTH and corticosterone. A study conducted by Baranowska et al. [14] found that maternal HFD has contributed to obesity and metabolic disorders in both male and female offspring during mating, pregnancy and lactation. The finding that male adult offspring from HFD dams were more obese compared to offspring from normal diet and treatment groups was confirmed in our research. As early as week 4 of age, male offspring on HFD had higher bodyweight.

However, at 6 weeks onwards of age, adult male offspring from dams treated with *E.tapos* shell (OTX2) had the lowest bodyweight among all groups. A recent research by Nor Liyana et al. [8] indicated that *E.tapos* shell is a potent source of natural anti-oxidant and has a high compound of flavonoids and phenols. Similarly, in this research, compared to seed extraction, *E.tapos* shell extract has better anti-obesity activity. Before pregnancy, treatment with *E.tapos* shell extraction can reduce the bodyweight of obese female rats. In the second generation of female rats, the anti-obesity effect of the *E.tapos* shell may be observed. The *E.tapos* seed and shell have also been reported to act as an inhibitor of pancreatic lipase [8]. Pancreatic lipase inhibition may decrease fat ingestion and thus energy absorption, which is one of the main targets thought to mediate obesity [15]. This study showed that obese dams will produce a high bodyweight offspring that leads to more complications in serious illnesses. Current research has shown that the DNC group fed witha 5-weeks of HFD and cafeteria diet have increased the body weight and adiposity of their male offspring. A comparable study by Santhra et al. [11] also shows that pre-pregnancy supplementation of *E.tapos* shell extraction among obese dams showed an anti-obesity effect on their offspring as early as postnatal day 21. This finding highlights the importance of human beings changing their way of eating and having a healthy lifestyle to generate a physically and mentally healthy new generation. In their reproductive age, supplementation of *E.tapos* among females will help cut off the obesity chain in the cycle of human life.

Higher cortisol concentrate have been causally related physiologically to fat build-up and weight increment, as glucocorticoids facilitate pre-adipocyte conversion to mature adipocytes. Research by Rebuffé-Scrive and colleagues proved that male Sprague-Dawley rats that have been stressed for 28 days had significantly enlarge dipocytes than controls and has a propensity to show a heavier abdominal wall fat pad [16]. This finding was consistent with our findings with the male offspring from HFD dams had higher total body weight, ACTH and corticosterone plasma level as compared to male offspring from normal diet dams group. A similar study by Buchenauer et al. [17] showed that obese rats had a significantly higher corticosterone level as compared to lean rats. Cortisol also drives insulin resistance via multification of adipocytokines and the secretion of pro-inflammatory cytokines. Stress-related cortisol concentrations play a significant role in adipocyte biology and weight gain, potentially implicating it as a key component in the development of obesity [18]. In our research, increased level of plasma ACTH and corticosterone in offspring from HFD dams has been ameliorated by the supplementation of *E.tapos* seed and shell. This was showed by the lower level of ACTH and corticosterone in male adult offspring from dams supplemented with *E.tapos* seed and shell compared to offspring from HFD group. Previous research used obese Zucker rats found that 11β-HSD1 was elevated in adipose cells, probably enhancing the initiation of local glucocorticoids and thus stimulating obesity. Other research, in rodent models of obesity of various etiologies, have mostly confirmed increased 11β-HSD1 activity and mRNA levels selectively in fat tissue but not in the hepar. Genetically modified mouse selectively over-expressing 11β-HSD1 in fat tissue had features of visceral obesity and chronic diseases such as diabetes mellitus, hyperlipidemia and high blood pressure [18]. *E.tapos* seed and shell possibly act in deactivation of corticosterone by suppressing the 11β-HSD1 expression.

Therefore, this research has proved that *E.tapos* seed and shell supplementation in obese dams prior to pregnancy has the potential to alleviate the stress hormone in their offspring. Data from this

study suggested that *E.tapos* seed and shell extraction may attenuate the corticosterone and ACTH level, therefore reduce stress among obese rats thus reduced fat accumulation and weight gain. In order to augment the success of *E.tapos* weight-loss treatments and ameliorate the stress hormone, suggested further research are to analyze the effect of *E.tapos* on the hormone of the hypothalamic-pituitary-adrenal (HPA) axis to investigate the effect of *E.tapos* in controlling stress hormone which commonly caused obesity, also memory, and cognitive deficit in mother and their offsprings.

5. Conclusions

In male adult offspring rats, diets with high fat content lead to obesity and metabolic disorders. Pre-pregnancy *E.tapos* extraction supplementation in female rats has been shown to decrease obesity in male offspring, as well as ameliorate the stress hormone level.

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