

# *Elacteriospermum tapos* Ameliorates Maternal Obesity Effect on Serum Leptin Changes in Male Offspring <sup>†</sup>

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**Abstract:** The purpose of this study is to investigate the effect of *Elateriospermum tapos* aqueous extract supplementation on serum leptin of male offspring at weaning. Total 30 female Sprague Dawley rats were assigned to 2 groups where control group (CG) consist of 6 rats and the remaining rats were induced obesity for five weeks with a high-fat diet pellet and cafeteria food. After five weeks, the obese group further divided into four groups, negative control group (NG), positive control group (PG) (orlistat 200 mg/kg), treatment 1 (TX1) (200 mg/kg *E.tapos* seed) and treatment 2 (TX2) (200 mg/kg *E.tapos* shell) for 6 weeks. After six weeks, all rats were mated and continue with their respective diet till weaning. One male pup from each dam culled at weaning [postnatal day 21 (PND21)]. Results shown bodyweight in male offspring (M) from negative group dams (NG) had significantly heavier as compared to other pups groups. Total adipose tissue weight in MTX1 and MTX2 of the male offspring also show significantly lower compare to MNG. In mums, serum leptin of NG shows significantly higher as compared to CG group. Whereas, both treatment groups showed a significant reduction in serum leptin compared to NG group. In pups, MTX2 group show a more substantial reduction of body weight and serum leptin compared to other pups from other mums groups. In conclusion, *E.tapos* aqueous extract supplementation has a greater effect on ameliorating maternal obesity effects on male offspring by lowering body weight, inhibit fat deposition, and reducing serum leptin.

**Keywords:** maternal obesity; *Elateriospermum tapos*; high-fat diet; cafeteria diet; leptin

## 1. Introduction

Maternal obesity is a contributor to childhood obesity because it does not only increase the number of children who are overweight or obese. It also increases the potential risk of children suffering from diseases including type 2 diabetes, cardiovascular diseases, asthma, dyslipidaemia, sleep apnea, non-alcoholic fatty liver disease, orthopaedic problems, depression and low self-esteem on their early childhood [1]. Later, a lot of overweight children becoming adult obese and expose themselves to a greater risk of chronic diseases and disabilities. From looking at cost perspectives, diagnosis of obesity results in greater use of services as well as higher healthcare costs [2]. Diseases start earlier when a child is obese and often last until adulthood, with predictable implications of lost productivity, missed working days, healthcare costs increased, quality of life decreased, and lifespan is shortened [3]. Public awareness and knowledge on maternal obesity and its outcomes towards childhood obesity are yet low, and the public neglects it.

Leptin is a 16 kDa protein hormone that produces by the Ob (Lep) gene. Leptin plays a crucial primary role in the regulation of the hypothalamus pituitary-gonadal axis mainly on feeding circulatory system focusing on body mass, energy balance and activity level—the primary site of leptin production on adipose tissue. The previous study shows that the leptin mRNA in adipocytes correlated with body weight, which is mainly due to the ob gene [4,5]. If the adipocyte cell is more significant, the amount of leptin produced is will increase and vice versa. The study also reveals that leptin expression also can be found in non-adipose tissue such as stomach, muscle and placenta [4,5].

Therefore, there is a need to address proper care and disease management of obese in pregnant women using alternatives such as natural remedies or traditional medicines. Preventive measures using alternative medicine such as *Elateriospermum tapos* (*E.tapos*) could be very significant in combating obesity, especially towards the offspring. This research attempts to identify alternative medicine that is effective to decrease the prevalence of children suffering from childhood obesity and any comorbidities.

## 2. Materials and Methods

### 2.1. *Elateriospermum tapos* Plant Identification

The *E. tapos* was collected from Maran Pahang, and the sample was sent to the Herbarium Biodiversity Unit (UBD) at UPM for identification (UPM SK 3154/17).

### 2.2. *Elateriospermum tapos* Extraction

The hot aqueous method was used to obtain the *E.tapos* powder from shell and seed. As general 50 g of *E.tapos* shell and seed weighs using the digital weighing machine and this well grind *E.tapos* shell/seed mix with 500 mL distilled water in difference Scott bottle. Both Scott bottle was placed in a water bath for 70 °C for 24 h. The solution was filtered after 24 h using Whatman paper No 1. The later undergo the freeze-drying process to obtain a powder. *E.tapos* shell and seed powder keep in flacon tube and store under – 20 °C [6].

### 2.3. Induction of Obesity

The rats were given a high-fat diet (HFD) made of 43% carbohydrate, 17% proteins, and 40% fats (414 kcal/100 g). HFD was prepared using 68% of standard rat pallet (Gordon Specialty Stockfeed, Malaysia), 6% corn oil (Vecorn brand), 20% milk powder (Dutch lady) and 6% ghee (Crispo brand). All the ingredients were mixed using blander, and this mixture baked in the oven at 60 °C for 2 h. The baked HFD later cut into small pieces and keep in the freezer. This HFD was given to all obese group rats for five-week to induced the obesity together with cafeteria food (CF) such as marble cake (440 kcal/100 g), beef sausage (260 kcal/100 g), and savoury snacks (566 kcal/100 g) [6].

#### 2.4. Animal Experimental Study Design

All procedure involving animal work was conducted under the approval of the Animal Care and Ethics Committee of Management and Science University, AE-MSU-073. Total of thirty female ( $n = 30$ ), young Sprague Dawley rats, weighing between 150 g–200 g was used in this research. Upon received the rats, all rats were acclimatized for one week and allowed them to free for food and water access. Later these rats were divided to control group (6 rats) (CG) fed with standard chow. Remaining twenty-four rats were given with high-fat diet (HFD) and selected cafeteria food (CF) for five weeks to generated obesity. After confirmation on obesity among HFD group compared to CG, HFD group rat was separated to 4 different groups (each group with  $n = 6$  rats), negative control group (NG), positive control group (PG) with Orlistat administration (200 mg/kg), treatment with *E.tapos* seed (TX1) (200 mg/kg) and also with *E.tapos* shell (TX2) (200 mg/kg). Treatment for all rats was conducted for six weeks before introduced these rats with male rats for mating purpose. Within two days of birth, the number of each group was adjusted to 8–12 pups only. Pups were weighed every two days until weaning postnatal day (PND21) and also until week 13 using an electronic scale.

#### 2.5. Plasma Biochemistry

Five milliliter blood volume was collected through cardiac puncture and place in heparin tubes and centrifuged at 2500 RPM for 15 min to obtain plasma. Plasma leptin concentration was measured using commercially available ELISA kits (Sigma-Aldrich, St. Louis, MO, USA, RAB0335) according to the manufacturer's instruction.

#### 2.6. Statistical Analyses

SPSS 25.0 Windows software was used to analyses the statistical data and results were expressed as mean  $\pm$  SEM. A normality test was done for all the data. Serum leptin data of dams after treatment was 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.

### 3. Result and Discussion

#### 3.1. Body Weight and Total Adipose of Male Pups (PND21)

A significant increase ( $p < 0.05$ ) of body weight was observed among MNG group pups compare to the MCG group. MPG group, which treat with Orlistat, shows a significant reduction of body weight compared to the negative control. The treatment group with *E.tapos* seed and shell also show a considerable reduction compare ( $p < 0.05$ ) to the positive control group (Table 1).

The weight of total adipose tissue for MNG shows a 64.7% increase compared to the MG group. Treatment group MPG, MTX1, and MTX2 show no significant ( $p > 0.05$ ) reduction on total adipose tissue than the MNG group. However, the percentage of reduction show 11.67% for MPG, 26.14% for MTX1, and 28.11% for MTX2.

The previous study shows that the *E.tapos* seed and shell have a high level of flavonoid, specifically 3'4'5' Trimethoxyflavone that has nutritional properties. Study shows that the bodyweight rat feed with this *E.tapos* seed or shell reduce body weight and also the fat level in the rat, due to flavonoid show as good in reducing fat accumulation in the rat by increase the beta-oxidation of fatty acids. Top of that, it also reduced the total adipose tissue weight among *E.tapos* treated group [6–9].

Among *E.tapos* seed and shell, the shell has more effective and significant effect compared to seed even though both have flavonoid compound. It shows that *E.tapos* shell has more flavonoid compared to a seed. In present study show that body weight and total adipose tissue weight in *E.tapos* shell treated group showed more significant effect compared to a seed. It shows that *E.tapos* shell contains flavonoids which help to alleviate the fat oxidation by removed the storage of fat in rat [6–9].

**Table 1.** Body weight and total adipose weight of male pups (PND21).

	MCG	MNG	MPG	MTX1	MTX2
Body weight (g)	40.94 ± 4.00 <sup>b</sup>	50.00 ± 2.30 <sup>a</sup>	42.11 ± 2.07 <sup>a</sup>	37.34 ± 1.37 <sup>a</sup>	34.30 ± 1.48 <sup>a,c</sup>
Total adipose weight (g)	0.54 ± 0.13	1.53 ± 0.35	1.37 ± 0.62	1.13 ± 0.30	1.10 ± 0.11

Abbreviations: Total adipose tissue represents the sum of RP, Visceral, and gonadal fat mass. The first letter indicates male offspring (M); the second letter indicates the groups. MCG; offspring control group; MNG, offspring positive control; MPG, offspring positive group, MTX1, offspring treatment 1 group and MTX2, offspring treatment 2 group. Data are expressed as mean ± SEM and were analyzed by one way ANOVA, followed by post-hoc LSD. Significant level set at  $p < 0.05$ . <sup>a</sup>  $p < 0.05$  versus negative control, <sup>b</sup>  $p < 0.05$  versus normal control, <sup>c</sup>  $p < 0.05$  versus positive control.

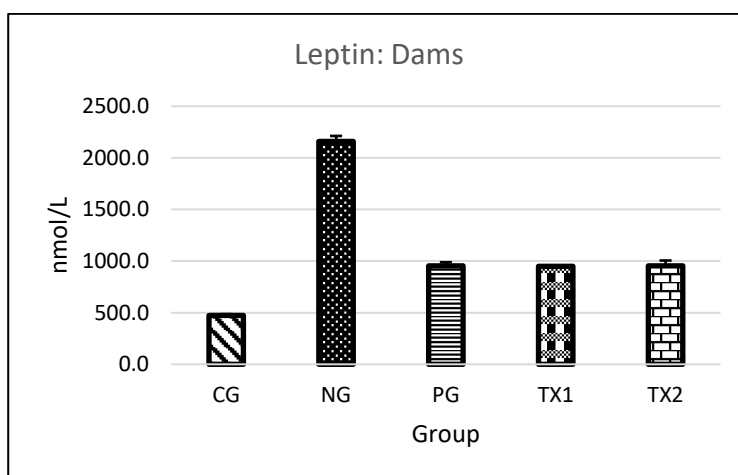
### 3.2. Serum Leptin of Dams and Male Pups (PND21)

Figure 1 shows the leptin concentration level of dams in serum. The NG group's serum leptin concentration level was significant ( $p < 0.05$ ) higher than the CG group. Intact PG, TX1, and TX2 serum leptin concentration show significant ( $p < 0.05$ ) reduction compare to the NG group. Figure 2 shows the leptin concentration level of male pups at PND21. The serum leptin of male pups from negative group dams (MNG) show a similar trend as dams. MNG's serum level concentration shows a significant increase ( $p < 0.05$ ) compared to MNG pups. Pups from MPG, MTX1, and MTX2 show a significant reduction ( $p < 0.05$ ) compare to the MNG group. This also shows the same trend as the dams group.

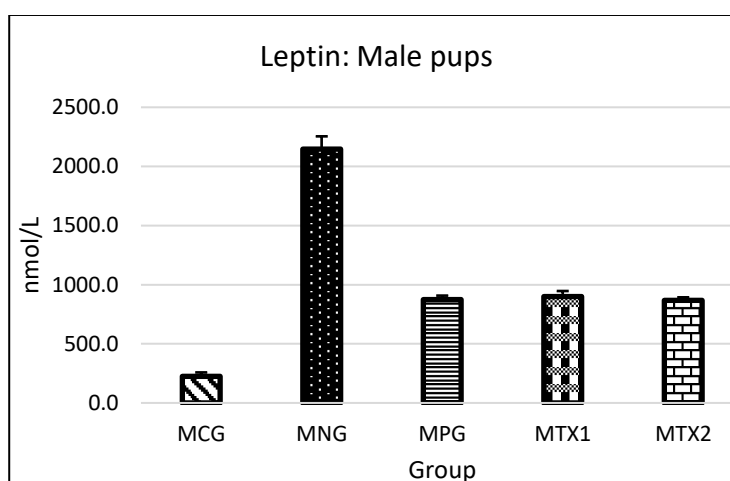
Overall, there was a positive correlation between body weight of dams and leptin concentration level in dams ( $r = 0.323$ ,  $n = 30$ ,  $p = 0.082$ ). However, for male pups significant positive correlation show between body weight and pup leptin concentration level ( $r = 0.427$ ,  $n = 30$ ,  $p = 0.019$ ). It show increase in body weight were correlated with increases in leptin concentration level in dams and pups.

The increasing of serum leptin concentration level in rats directly correlated with the bodyweight of rats. More fat accumulation in the body, the concentration of leptin also will be high in plasma or serum of rats. The previous study conducted by Fangyan Du et al. (2000), shows that rat fed with high-fat diet reveal increasing of leptin level compare those not provided with a high-fat diet. Leptin know as one of the feedback signals from fat reduces and increases thermogenesis. This brings the general conclusion that leptin is mainly involved in the regulation of energy balance in the body [5]. The previous study also shows that rat fed with a high-fat diet have resistance toward to leptin. This is due to the high-fat diet-fed rat have saturation or defect of the leptin, especially in transport system across the blood-brain barrier. This will cause in balance on energy intake and also energy expenditure in rats [5,10].

The current study shows that there is a positive correlation between body weight and leptin level. Obese dams have a high level of serum leptin compare to *E.tapos* treated group dams. Proportionally it shows the fat level, and the bodyweight of this group reduce and lead to a decrease in leptin concentration level. A similar result has shown in male pups at PND21 which is pups from obese dam show a high level of serum concentration leptin compared to *E. tapos* treated group pups. The current study indicates that *E.tapos* shell has more effective compared seed to help reduce the body weight, adipose accumulation and also the leptin level as well.



**Figure 1.** Impacts of *E. tapos* on serum leptin of dams. Abbreviations: CG; control group, NG, negative control, PG, positive group, TX1, treatment with seed, TX2, treatment with shell. Data are expressed as mean± SEM and were analyzed by one way ANOVA, followed by post-hoc LSD.



**Figure 2.** Impacts of *E. tapos* on serum leptin of male pups (PND21). Abbreviations: the first letter indicate male offspring (M); the second letter indicates the groups. MCG; offspring from control group, MNG, offspring negative control, MPG, offspring positive group, MTX1, offspring from dams treated with seed group and MTX2, offspring from dams treated with shell group. Data are expressed as mean± SEM and were analyzed by one way ANOVA, followed by post-hoc LSD.

#### 4. Conclusions

As a conclusion, the effect of *E. tapos* seed and shell extraction showed a beneficial effect on reducing body weight, total adipose tissue weight and also serum leptin concentration level. However the *E. tapos* shell extraction shows a more prominent effect as an anti-obesity supplement as compared to *E. tapos* seed extraction.

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