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Elacteriospermum tapos Ameliorates Maternal Obesity Effect on Serum Leptin Changes in Male Offspring †

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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. A total of 30 female Sprague Dawley rats were assigned to two groups, where the control group (CG) consisted of six rats and the remaining rats had obesity-induced over five weeks with a high-fat diet pellet and cafeteria food. After five weeks, the obese group was further divided into four groups, a negative control group (NG), positive control group (PG) (orlistat 200 mg/kg), treatment 1 (TX1) (200 mg/kg BW of *E. tapos* seed) and treatment 2 (TX2) (200 mg/kg BW of *E. tapos* shell) for 6 weeks. After six weeks, all rats were mated and continued with their respective diet till weaning. One male pup from each dam culled at weaning (postnatal day 21 (PND21)). The results show that body weight in male offspring (M) from negative group dams (NG) was significantly heavier as compared to other pup groups. Total adipose tissue weight in MTX1 and MTX2 of the male offspring was also significantly lower compared to MNG. In mums, serum leptin of NG was significantly higher as compared to the CG group, whereas both treatment groups showed a significant reduction in serum leptin compared to the NG group. In pups, the MTX2 group showed a more substantial reduction in body weight and serum leptin compared to other pups from other mother's 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 not only does it 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 in early childhood [1]. Later, a lot of overweight children become obese adults and expose themselves to a greater risk of chronic diseases and disabilities. From the cost perspective, the 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 the predictable implications of lost productivity, missed working days, increased healthcare costs, decreased quality of life, and a shortened lifespan [3]. Public awareness and knowledge on maternal obesity and its outcomes towards childhood obesity are low, and it is neglected by the public.

Leptin is a 16 kDa protein hormone that is produced by the Ob (Lep) gene. Leptin plays a crucial primary role in the regulation of the hypothalamus pituitary–gonadal axis, mainly in the feeding circuits focusing on body mass, energy balance and activity level—the primary site of leptin production in adipose tissue. The previous study shows that the leptin mRNA in adipocytes was 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 will increase and vice versa. The study also reveals that leptin expression can also be found in non-adipose tissue such as the stomach, muscle and placenta [4,5].

Therefore, there is a need to address the proper care and disease management of obesity 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 significant in combating obesity, especially in offspring. This research attempts to identify alternative medicine that is effective in decreasing the prevalence of children predisposed to childhood obesity and any comorbidities.

2. Materials and Methods

2.1. Elateriospermum tapos Plant Identification

The *E. tapos* was collected from Maran Pahang, Malaysia 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. Approximately 50 g of *E. tapos* shell orseed was weighed separately using the digital weighing machine, and this was well-grounded into powder and shell or seed was mixed with 500 mL distilled water in a different Scott bottle. Both Scott bottles were placed in a water bath at 70 °C for 24 h. The solution was filtered after 24 h using Whatman paper No 1. Next, the filtrate underwent the freeze-drying process to obtain an extract powder. *E. tapos* shell and seed powder were kept in a flacon tube and stored at 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 (Gold Coin Feedmills (M) Sdn Bhd, Selangor, Malaysia), 6% corn oil (Vecorn, Yee Lee Corporation Berhad, Kuala Lumpur, Malaysia), 20% milk powder (Dutch lady, Dutch Lady Milk Industries Berhad, Selangor, Malaysia) and 6% ghee (Crispo, Crispo-Tato (M) Sdn Bhd, Kuala Lumpur, Malaysia). All the ingredients were mixed and baked in the oven at 60 °C for 2 h. The baked HFD was later cut into small pieces and kept in the freezer. This HFD was given to all obese group rats for five weeks to induced 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. A total of thirty female (n = 30), young Sprague Dawley rats, weighing between 150 and 200 g, were used in this research. Upon receiving the rats, all rats were acclimatized for one week and allowed free food and water access. Later, these rats were divided into the control group (six rats) (CG), fed with standard chow. The remaining twenty-four rats were given a high-fat diet (HFD) and selected cafeteria food (CF) for five weeks to generate obesity. After confirmation of obesity among the HFD group compared to CG, HFD group rats were separated into four different groups (each group with n = 6 rats): negative control group (NG), positive control group (PG) with Orlistat administration (200 mg/kg BW), treatment with *E. tapos* seed (TX1) (200 mg/kg BW) and treatment with *E. tapos* shell (TX2) (200 mg/kg BW). Treatment for all rats was conducted for six weeks before introducing these rats to male rats for mating purposes. 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 placed 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 ± SEM. A normality test was done for all the data. Serum leptin data of dams after 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.

3. Result and Discussion

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

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

The weight of total adipose tissue for MNG showed a 64.7% increase compared to the MG group. Treatment groups MPG, MTX1, and MTX2 showed no significant ($p > 0.05$) reduction in total adipose tissue compared to the MNG group. However, the percentage of reduction was 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, which has nutritional properties. The study shows that the body weight of rats fed with this *E. tapos* seed or shell was reduced, as were the fat levels in the rat, due to flavonoids, which were shown to be good at reducing fat accumulation in the rat by increasing the beta-oxidation of fatty acids. Furthermore, it also reduced the total adipose tissue weight among the *E.tapos*-treated group [6–9].

Among *E. tapos* seed and shells, the shell has a more significant effect compared to seed, even though both have the flavonoid compound. This shows that the *E. tapos* shell has more flavonoid compared to a seed. The present study shows that body weight and total adipose tissue weight in the *E.tapos*-shell-treated group had a more significant effect compared to a seed. This shows that *E. tapos* shell contains flavonoids, which help to alleviate the fat oxidation by removing the storage of fat in rats [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 [b]	50.00 ± 2.30 [a]	42.11 ± 2.07 [a]	37.34 ± 1.37 [a]	34.30 ± 1.48 [a,c]
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. Significance 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.

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 significantly higher ($p < 0.05$) than the CG group. Intact PG, TX1, and TX2 serum leptin concentration show a significant reduction ($p < 0.05$) compared 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) shows a similar trend to 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 ($p < 0.05$) compared 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, a significant positive correlation was shown between body weight and pup leptin concentration level ($r = 0.427$, $n = 30$, $p = 0.019$). This shows that increases in body weight were correlated with increases in leptin concentration level in dams and pups.

The increasing serum leptin concentration level in rats directly correlated with the body weight of rats. If there is more fat accumulation in the body, the concentration of leptin also will be high in the plasma or serum of rats. The previous study, conducted by Fangyan Du et al. (2000), shows that rats fed with a high-fat diet reveal increasing leptin levels compared to those not provided with a high-fat diet. Leptin, known 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 rats fed with a high-fat diet have resistance to leptin. This is due to the high-fat diet-fed rats having saturation or defects in the leptin, especially in the transport system across the blood–brain barrier. This will cause an imbalance in 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 compared to the *E.tapos*-treated group dams. Proportionally, this shows that the fat level and the bodyweight of this group reduces and leads to a decrease in leptin concentration level. A similar result has been shown in male pups at PND21, in which pups from an 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 is more effective compared to seed in helping to reduce the body weight, adipose accumulation and also the leptin levels.

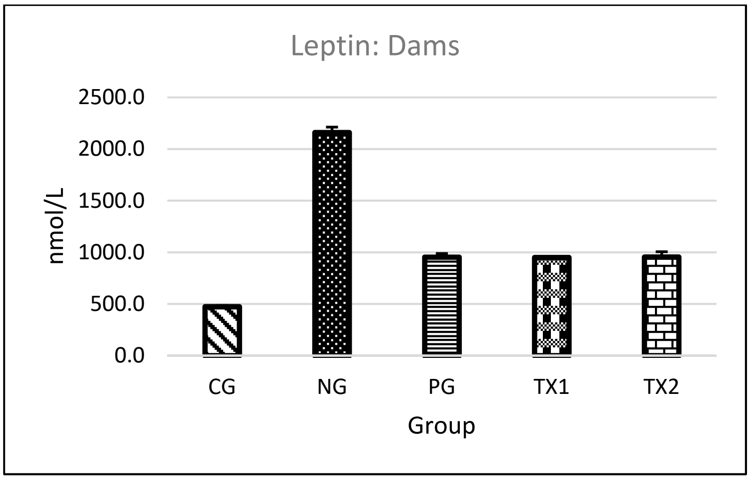

Figure 1. Impacts of *E. tapos* on serum leptin of dams. Abbreviations: CG; control group, NG, negative control, PG, positive group, TX1, treatment with seed, TX2, treatment with shell. Data are expressed as mean± SEM and were analyzed by one-way ANOVA, followed by post-hoc LSD.

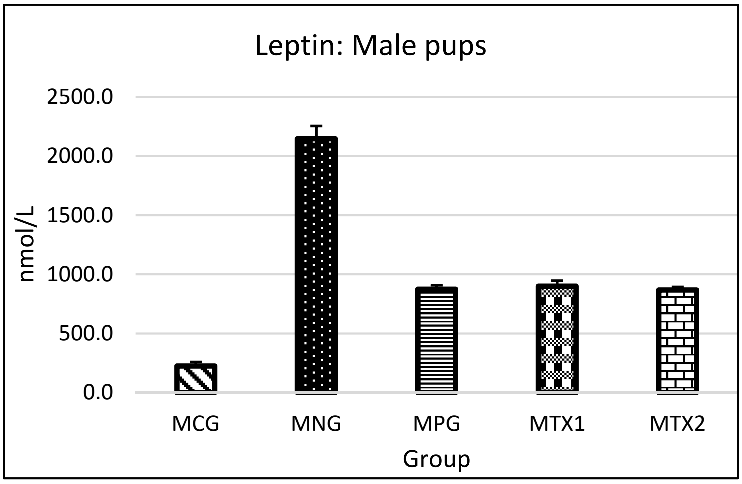

Figure 2. Impacts of *E. tapos* on serum leptin of male pups (PND21). Abbreviations: the first letter indicate male offspring (M); the second letter indicates the groups. MCG; offspring from control group, MNG, offspring negative control, MPG, offspring positive group, MTX1, offspring from dams treated 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, *E. tapos* seed and shell extraction had 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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