Int J Clin Exp Physiol. 2022;9(1):28-32
www.ijcep.org

Original Article

Effect of *Moringa oleifera* Consumption during Lactation and Early Post-weaning Period on Lipid Profile and Diet-induced Programming of Obesity in the Offspring of Wistar Rats

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ABSTRACT

Background and Aim: The fact that sub-optimal environments *in utero* and early postnatal alters the development and predispose to lifelong health problems, which has been proven by a litany of human epidemiological and animal laboratory studies. This study was designed to investigate whether or not maternal consumption of aqueous extract of *Moringa oleifera*, during lactation will affect the metabolic consequences of litter-reduced and litter-expanded offspring later in life. **Materials and Methods:** Thirty-two pregnant Wistar rats were used for this study. These rats were divided into two groups at delivery with each consisting of 16 rats. In group 1, the litters were reduced to four litters per dam to simulate over-nutrition while in group 2, litters were increased to eight litters per dam to simulate mild under-nutrition of the offspring. Each group was subdivided into 4 subgroups consisting of 4 dams in each subgroup. One group served as control group and were administered only water while other 3 groups were given 200mg/kg, 400mg/kg and 800mg/kg body weight of the extract respectively. Offspring food and fluid intake from weaning to postnatal day 42, glucose tolerance test and lipid profile on postnatal day 42 were determined. **Results:** The extract of *Moringa oleifera* significantly improved the lipid profile of both subgroups 1 and 2 offspring. **Conclusion:** Administration of aqueous leaf extract of *Moringa oleifera* during lactation had a lipid lowering effect on the offspring and may also protect against lipid derangement in the offspring later in life.

Key words: Estrus cycle, Lipid profile, Glucose tolerance test, Litters, *Moringa oleifera.*

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History

- Submission Date: 08-01-2022;
- Review completed: 19-02-2022;
- Accepted Date: 04-03-2022.

DOI : 10.5530/ijcep.2022.9.1.5

Article Available online
http://www.ijcep.org/v9/i1

INTRODUCTION

Obesity occurs when there is a chronic imbalance between energy intake and expenditure. Therefore disorders in the systems which regulate energy homeostasis can lead to obesity and related metabolic complications.[1] Worldwide, obesity and related metabolic disorders are considered as a major health challenge and epidemiological data suggests that susceptibility to these diseases depends on genetic, dietary and lifestyle. Changes in lifestyle associated with sedentary habits and consumption of foods rich in energy is implicated to be the major cause of obesity.[2] Many medications have been used to manage obesity over the years but most of these drugs which were approved and marketed have now been withdrawn due to serious adverse effects.[3] Due to dissatisfaction with high costs and potentially hazardous side effects associated with these drugs, alternative strategies employing the use of natural products to provide safe treatment of obesity are being explored. These include crude extracts and isolated compounds from plants which can induce body weight reduction and prevent diet induced obesity.[4] Moringa (*Moringa oleifera* Lam) is a native of the western and Sub Himalayan tracts, India, Pakistan, Asian Minor, Africa, and Arabia. It is now distributed in the Philippines, Cambodia, Central America, North and South America and Caribbean Islands.[5] It is also called the Horseradish Tree, Sajna, Keler, Benzolive, Mlonge etc. It is shown in scientific division to come from Kingdom: Plantae, Division: Magnoliphta, Class: Magnolipsida, Order: Brassicales, Family: *Moringaceae*, Genus: Moringa, Species: *M. Oleifera*.[6] In traditional/folklore medicine, different parts of this plant are used for the treatment of a variety of human ailments.[4] *Moringa oleifera* is known to possess anti- inflammatory, anti-spasmodic, anti-hypertensive, anti-tumour, antioxidant, anti-pyretic, anti-ulcer, anti-epileptic, diuretic, cholesterol lowering, renal, anti-diabetic, and hepato-protective activities.[6] Therefore, due to its healing abilities for various ailments and even some chronic diseases, it earned the name 'the miracle tree'.[7] There is paucity of data on the effect of M. oleifera extract on the development of obesity in

Cite this article: Okeke AP, Ani CO, Nweke ML, Agbor JI, Okolo KO, Ndubuisi NR, *et al.* Effect of *Moringa oleifera* Consumption during Lactation and Early Post-weaning Period on Lipid Profile and Diet-Induced Programming of Obesity in the Offspring of Wistar Rat. Int J Clin Exp Physiol. 2022;9(1):28-32.

the offspring. The present study was designed to investigate this. Obesity and its related metabolic disorders is a growing concern worldwide with an alarming prevalence of 8.1% -22.2% in Nigeria.[8]

Though there are drugs that can be used to treat full blown obesity and its related metabolic disorders, they are not 100% effective and they also have adverse side effects. In general, management of full-blown obesity is an expensive venture and so cheaper means of treatment and prevention have to be explored. In line with the DoHaD hypothesis, the 'blueprints' of future diseases are laid during the developmental periods of pregnancy and lactation. Hence the modern trend now in the scientific world is the development of strategies that can prevent the expression of these developmentally programmed diseases. Since it is well established that over nutrition by litter reduction predisposes to the development of obesity later in life, the present study was designed to investigate whether or not maternal consumption of aqueous leaf extract of *M. Oleifera* (ALEMO), a wonder plant used locally in the management of a variety of ailments, during lactation and the early post-weaning period will prevent or reduce the development or severity of the programmed obesity and its related metabolic disorders later in life. The aim of the present study was to investigate the effect of *M. Oleifera* consumption during lactation and early post-weaning period on diet-induced programming of obesity in the offspring.

MATERIALS AND METHODS

Plant Collection, Identification and Authentication

The fresh leaves of *Moringa oleifera* were collected from a flower garden in Abuja metropolis, Nigeria. A sample of the leaf of this plant was identified and authenticated at the herbarium section of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. A voucher specimen was deposited in the herbarium for further reference with no (UNH 16C). The leaves were air dried at room temperature (25±0.1°C) and reduced to coarse powder using a mechanical grinder. The extraction procedure outlined by Ani *et al*., 2017 was applied.[9] The *Moringa* flower powder was processed using distilled water, the mixture was vacuum filtered using Whatman No. 1 filter paper and concentrated using water bath at a temperature of 60°C and the yield was saved in a glass container and stored in a refrigerator pending use.

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times 100$$

$$\frac{329.695}{1778.357} \times 100$$

% Yield= 18.54%

Experimental Procedure

Thirty-two matured inbred non-pregnant female mice were procured from the Animal House of the Department of Physiology, University of Nigeria, Enugu Campus for this study. These animals were housed in stainless steel and well-kept in ventilated cages under controlled environmental conditions. The animals were provided standard commercial pelleted feed (Vital feed, Nigeria) and water *ad libitum.*

Ethical Clearance

Ethical clearance for this study was obtained from the Health Research Ethics Committee, University of Nigeria Teaching Hospital, Enugu.

Determination of Estrus Cycle

This was done according to the method of Hamid and Zakaria, 2013.[10] The estrus cycle was monitored for each mouse by examining the vaginal smears at fixed times daily under light microscopy and mice with two consecutive regular four-day estrus cycle was used for this study. At pro-estrus, male mice were introduced into the female cages in the ratio of 1:2 to allow for mating. The presence of sperm in the vaginal smear or observation of a vaginal plug indicated the occurrence of mating. Day 1 of gestation was considered as the day the sperm was seen in the vaginal smear.[10] From day 1 of pregnancy to delivery (i.e throughout pregnancy) mice were given food and water *ad libitum.*

On the day of delivery, the dams were randomly divided into two main groups; Group 1 and Group 2 consisting of 16 dams each.

In group 1, the litters were reduced to four offsprings per dam to simulate over-nutrition of the offspring while in group 2 litters were increased to eight litters per dam to simulate under-nutrition of the offspring.[11]

Experimental Design

Each of these groups was divided into 4 subgroups consisting of 4 dams in each subgroup as shown below;

Group A- served as control group and was administered water only.

Group B- These dams were given low dose (200mg/kg) of the extract.

Group C- These dams were given medium dose (400mg/kg) of the extract.

Group D- These dams were given high dose (800mg/kg) of the extract

Determination of Fluid and Food Intake

Every morning, the same quantity of food was administered to all the pups from post-natal day 21 to 42 in their different cages. Each quantity of food and water administered was weighed and recorded. Before administration of the next meal, the left-overs were gathered and recorded and then subtracted from the original quantity that was administered the previous day and then recorded.[12]

Determination of Body Weight and Length

The body weights (g) of the pups were recorded on day one and then weekly consecutively for 42 days using a digital weighing balance.

Body length of the pups were also determined (crown-rump or naso–anal length) on day one and then weekly consecutively for 42 days.

The body weight and body length was then used to determine the Body Mass Index (BMI)

$$\text{BMI} = \frac{\text{Body weight (g)}}{\text{length (cm)}^2}.$$

Determination of Serum Lipid Profile

At post- natal day 42, the lipid profile of pups from each sub group was determined. Serum high density lipoprotein cholesterol (HDL-c) was determined using Randox laboratories (England) kit.

The serum total cholesterol was determined using Randox laboratories (England) kit based on enzymatic endpoint method.

Serum triglyceride was determined using Hi-tech diagnostic kit. The low-density lipoprotein level was calculated from the value of serum HDL-c, cholesterol and triglyceride level.[13]

Determination of Oral Glucose Tolerance Test

This test was carried out using the procedure described by Ayala *et al*. (2010).[14] Prior to the test, the rats were fasted overnight. Blood was

obtained from a tail cut (by removing the distal 2 mm of the tail) and was assessed for baseline glucose levels using an Acucheck glucometer. The rats then received 2 g/kg body weight of a 100 mg/ml glucose solution in sterile water delivered by oral gavage.

At 30, 60, 90 and 120 min after the administration of glucose, dried blood and tissue were quickly removed from the tail wound and blood was collected again to measure the glucose concentration.

Ethical Approval

The ethical approval was obtained from the Faculty Ethics committee, Directorate of Research, College of Medicine, University of Nigeria, Enugu Campus with reference number UN22c.

Statistical Analysis of Data

The results were expressed as mean ± standard error of mean (SEM). For data comparison between the groups, one way analysis of variance (ANOVA) was used followed by a post-hoc student Newman-keuls test and p<0.05 was taken as statistically significant.

RESULTS

From Figure 1, it can be deduced that on the Day 0, over-nourished pups in the medium and high dose groups had significantly (P<0.05) lower BMI compared to the control, while on postnatal day 7, there was no significant (P>0.05) difference in BMI values in the over-nourished pups. On the postnatal day 14, over-nourished pups in the medium and high dose groups had significantly (P<0.05) greater BMI than those in the control group. Those in the high dose group had significantly (P<0.05) higher BMI than those in the medium dose group. On the Post-natal day 21, over-nourished pups in the medium dose group had significantly (P<0.05) greater BMI than those in the low dose group while those in the high dose group had significantly lower BMI than those in the medium dose group. On the post-natal day 28, over-nourished pups in the low dose group had significantly (P<0.05) greater BMI than the control. On post-natal day 35, it was observed that over-nourished pups in the high dose group had significantly lower BMI than those in the low dose. There was no significant (P>0.05) differences in BMI of over-nourished pups.

On the postnatal day 0, over-nourished pups in the high dose group had significantly greater BMI when compared to those in the medium dose group and when compared to their counterparts in the undernourished group, over-nourished pups in the medium and high dose groups had significantly (P<0.05) lower BMI on the postnatal day 7. Over-nourished pups in the control group had significantly (P<0.05) higher BMI values while those in the low and high dose groups had significantly lower BMI values. postnatal day 14, when compared to their counterparts in the undernourished group, over-nourished pups in the control, medium and high dose groups had significantly (P<0.05) greater BMI while on the post-natal day 21, when compared to their counterparts in the undernourished group, over-nourished pups in the low dose and high dose group had significantly (P<0.05) lower BMI while those in the medium dose group had significantly higher BMI. Post-natal day 28, when compared to their counterparts in the undernourished groups, over-nourished pups in the high dose group had significantly greater BMI. Post-natal day 35, there was significantly (P<0.05) greater BMI observed in the over-nourished pups in the control group compared to their counterparts in the undernourished group. Postnatal day 42 was compared to their counterparts in the undernourished group, over-nourished pups in the control, medium and high dose groups had significantly higher BMI values (Figure 2).

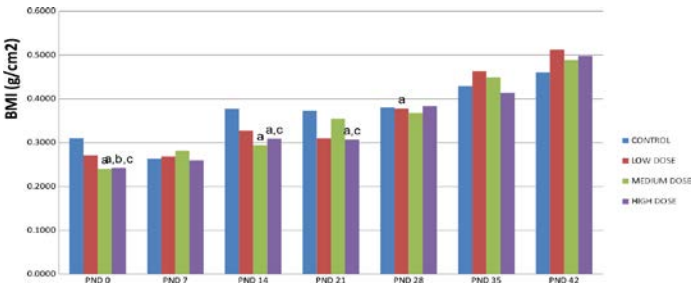

Figure 1: Effect of maternal administration of *m. oleifera* during lactation on BMI of over nourished pups (group 1).
Data represented as Mean ± SEM (Standard error of mean)

a=p<0.05 vs control b=p< 0.05 vs low dose c=p<0.05 vs medium dose

Table 1 shows that the food intake was highest in the group administered with medium dose of *m. oleifera* extract and this was followed by the control group while those that received a low dose of the extract consumed less amount of food compared to the control group 1 and the medium dose group. There was no statistical difference (P>0.05) between the low dose group and the medium dose group of the offspring between PND21 to PND42 compared to the normal control.

Table 2 depicts the effect of administration of aqueous leaf extract of *M. oleifera* during maternal lactation on weekly food intake of group 2. It was observed that the weekly food intake of the control group decreased

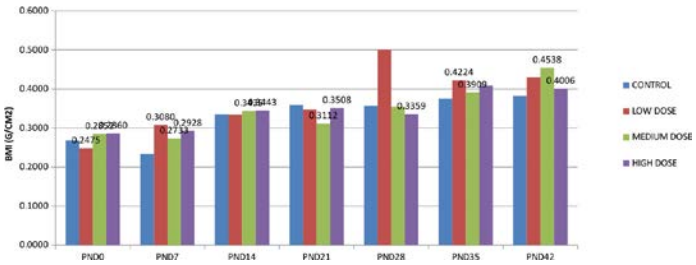

Figure 2: Effect of maternal administration of *m. oleifera* during lactation on BMI of undernourished pups (group 2).
Data represented as Mean ± SEM (Standard error of mean)

Table 1: Effect of administration of aqueous leaf extract of *m.oleifera* during maternal lactation on mean weekly food intake of offspring from PND 21- PND 42 in Group 1.

Groups	Week 1	Week 2	Week 3
Control	7.72±1.04	9.34±2.04	10.34±2.87
Low dose	5.02±0.36	7.60±0.53	8.94±0.19
Medium dose	7.87±1.09	8.39±0.05	11.83±0.34

Data represented as Mean ± SEM (Standard error of mean)

Table 2: Effect of administration of aqueous leaf extract of *M. oleifera* during maternal lactation on mean weekly food intake of offspring from PND 21- PND 42 in Group 2.

Groups	Week 1	Week 2	Week 3
Control	41.69±13.90	44.30±10.83	37.18±6.38
Low dose	19.00±1.15	19.86±2.61	31.80±4.59
Medium dose	17.00±1.75	21.82±2.68	27.07±3.05
High dose	30.17±6.37	29.59±7.73	33.03±4.62

Data represented as Mean ± SEM (Standard error of mean)

Table 3: Effect of administration of aqueous leaf extract of *M.oleifera* during maternal lactation on mean lipid profile of Group 1 offspring at PND 42.

Groups	LDL-C	HDL-C	TG	Total Cholesterol
Control	40.58±21.50	6.92±2.84	57.32±18.87	58.96±24.89
low dose	89.00±26.47	25.90±4.47[a]	141.44±26.99[a]	143.19±17.31[a]
medium dose	83.6±4.67b	35.44±3.01[a]	85.75±24.69	56.95±12.25[b]
high dose	85.07±28.72[c]	22.81±6.38[a]	106.86±15.59[a]	129.25±24.43[a,c]

Data represented as Mean ± SEM (Standard error of mean)

[a]=P<0.05 vs control; [b]=P < 0.05 vs low dose; [c]=P <0.05 vs medium dose

Table 4: Effect of administration of aqueous leaf extract of *M.oleifera* during maternal lactation on mean lipid profile of Group 2 offspring at PND 42.

Groups	LDL-C	HDL-C	TG	Total cholesterol
Control	30.24±5.45	11.40±4.00	68.37±19.21	60.91±12.89
low dose	57.06±15.80	12.45±4.91	73.15±6.44	84.15±11.04
medium dose	55.57±15.44	22.03±1.89[a]	119.47±36.12	101.49±21.13
high dose	56.82±23.41	14.00±4.74	94.02±12.98	89.62±21.91

Data represented as Mean ± SEM (Standard error of mean)

[a] =P<0.05 vs control

sequentially from week 1 to week 3 while there was an increase in the food intake for the low dose, medium dose and high dose groups respectively. It can also be deduced from the Table 3 that the food intake of high dose group was more significant in the high dose group when compared to the low dose and the high dose groups respectively. In spite the increase in the food intake of the test groups, there was no statistical significant difference (P>0.05) compared to the control group.

LDL-C concentration of the test groups were significantly (P<0.05) high compared to the normal control in the over-nourished group. The HDL-C was statistically significant (P<0.05) between the test groups and the control group at PND42. TG was statistically significant (P<0.05) difference between the low and high dose groups compared to the normal control group. There was also a significant increase in the total cholesterol concentration of the low dose and the high dose groups compared to the normal control group 1 offspring on PND42.

LDL-C concentration was increased in experimental groups than the normal control group in the under-nourished group. The HDL-C was also far less than the normal recommended reference range. Though the concentration was significantly (P<0.05) higher in the medium dose group compared to the normal control and this was followed by the high dose group and this did not follow a dose dependent pattern. Though TG and total cholesterol was increased in the experimental groups, there was no statistical significant (P>0.05) difference compared to the normal control (Table 4).

Figure 3 and 4 shows the effect of ALEMO on maternal lactation on OGTT of groups 1 and 2 offspring at post-natal day 42. There was no significant effect of the extract on fasting blood sugar levels and blood sugar levels 2hrs after glucose load in the over-nourished pups. However, when compared to the low dose group, the high dose group had significantly (P<0.05) higher fasting blood sugar levels which became lower after an hour of glucose administration followed by insignificantly lower blood sugar levels after another hour. This was observed in the undernourished pups.

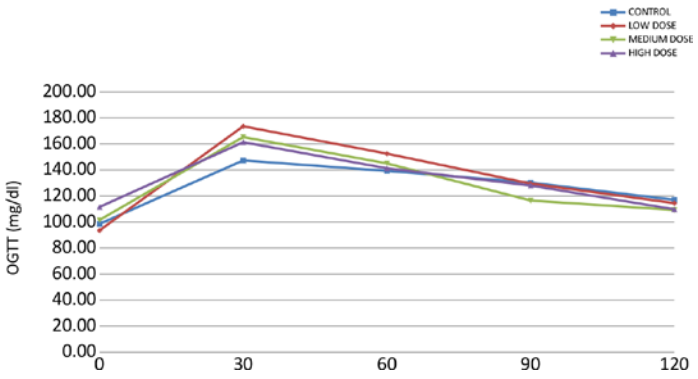

Figure 3: Effect of administration of aqueous leaf extract of *M. oleifera* during maternal lactation on OGTT of Group 1 offspring at PND 42.

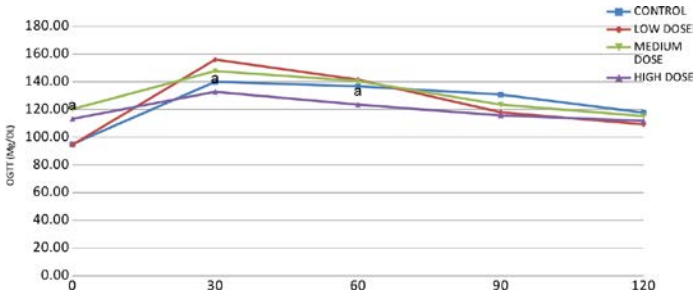

Figure 4: Effect of administration of aqueous leaf extract of *M. oleifera* during maternal lactation on OGTT of Group 2 offsprings at PND 42.
Data represented as Mean ± SEM (Standard error of mean)
[a] =P < 0.05 vs low dose

DISCUSSION

During lactation, from PND7-21, pups in group 2 (litter expanded group) had greater BMI compared with group 1 (litter reduced group) pups with extract treatment appearing to have a dose-dependent effect. These observations could be attributed to the fact that *Moringa oleifera* extract has been reported to have lactagogue effect. This effect of *Moringa oleifera* may be due to the presence of a compound called physosterol. Few studies shown that Moringa increases breast milk production and subsequently increases offspring weight gain.[15-16] It has been reported that the lactogenic effect of *M. oleifera* is due to increase in prolactin levels.[17] Prolactin is a lactagogue hormone that stimulates lactation. It has varied effects on the mammary glands namely; growth and development of the mammary gland (mammogenesis), synthesis of milk (Lactogenensis) and maintenance of milk secretion (galactopoiesis).[17] The suckling stimulus is the best known physiological stimulus which affects prolactin secretion and the amount of prolactin released is related to the intensity of the stimulus as it is somewhat commensurate with the number of pups being nursed.[17] This therefore implies that the more the number of pups being nursed, the greater the intensity of prolactin release and therefore the greater the breast milk production. This could account for the increased BMI seen in the pups in the 'undernourished groups' treated with the extract compared with the pups in the 'over-nourished group'. During post weaning, that is PND28-42, pups in group 2 had lower BMI compared with group 1 pups. It should be noted that during lactation, the pups in the over-nourished group (treated with extract with the exception of the control) were actually less fed when compared to their counterparts in the undernourished group.

This has already been attributed to the increased breast milk production facilitated by *M. oleifera* extract. The change in BMI observed from PND

28 may be due to a metabolic conflict in the pups. Studies have shown that childhood and adulthood obesity can be programmed during the developmental period and offspring who were subjected to nutritional deprivation in early life or were subjected to an environment rich in nutrients appear to be at risk.[18] Overfeeding during the suckling period in rodents by rearing them in small litters produce hyperphagia and obesity.[18] In the present study, extract administration significantly decreased the LDL-C and increased the HDL-C of group 1 (litter reduced) offspring whereas those of the group 2 (litter expanded) offspring were not affected. The reduction in LDL-C and cholesterol blood levels as well as the increase in HDL-C blood levels observed in the over nourished pups whose mothers were treated with the *Moringa oleifera* extract agrees with studies done by Aborhyem *et al*., 2016 and Saleem *et al*., 2016.[19-20]

CONCLUSION

They opined that *Moringa oleifera* effectively improved the atherogenic lipid profile in Wistar litter rats on lactation. No dose of the extract appeared to have any significant effect on the blood glucose levels of over-nourished pups both in the fasting state and 2 hrs after glucose loading. This could be due to the fact that *M. oleifera* extract played a role in the elevated blood sugar levels of all extract treated groups due to its lactogogue effect during lactation. Therefore all the groups had similar glucose tolerance levels. Another reason there seemed to be no significant effect is that the doses of the extract administered were not enough to counteract its initial prolactin-induced growth promoting effect as a result of increased breast milk production

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BMI: Body Mass Index; **LDL-C:** Low Density Lipoprotein Cholesterol; **HDL-C:** High Density Lipoprotein Cholesterol; **TG:** Triglyceride; **ALEMO:** Aqueous Leaf Extract of *M. Oleifera*; **OGTT:** Oral Glucose Tolerance Test; **PND:** Postnatal Day.

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Cite this article: Okeke AP, Ani CO, Nweke ML, Agbor JI, Okolo KO, Ndubuisi NR, *et al*. Effect of *Moringa oleifera* Consumption during Lactation and Early Post-weaning Period on Lipid Profile and Diet-Induced Programming of Obesity in the Offspring of Wistar Rat. Int J Clin Exp Physiol. 2022;9(1):28-32.