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The Impact of Ethanolic Extract of *Moringa oleifera* Leaf on Foetal Development and Growth in Streptozotocin-Induced Diabetic Wistar Rat.

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ABSTRACT

Diabetes mellitus is one of the unfavourable intrauterine environments in which foetus grow and this predisposes them to various abnormalities. The aim of this study was to investigate the impact of *Moringa oleifera* extract on foetal growth parameters in streptozotocin-induced diabetic wistar rats. Thirty (30) female Wistar rats (150-250g) were used. They were grouped into: group A (control), group B (diabetic), group C (Insulin), group D-E (diabetic treated 120mg, 240mg 600mg/kg *Moringa oleifera* respectively). The female animals were induced with diabetes (45mg/kg), mated with males in a 2:1 ratio. The presence of spermatozoa in the vaginal smear the following morning confirmed mating. The animals were sacrificed on day 20. The whole foetus was removed from the uterine horn and the weight, crown rump length and abdominal circumference were measured. The result revealed significant changes in the weight, crown rump length and abdominal circumference of all the animals in the *Moringa oleifera* treated groups.which could be attributed to its phytochemical content. This study has established that *Moringa oleifera* leaf extract can reverse the effect of maternal diabetes on the growth and development of foetus which could be attributed to its nutritious content.

Keywords: Diabetes mellitus, *Moringa oleifera*, foetus, development

INTRODUCTION

Diabetes in pregnancy is one of the commonest maternal illness which leads to congenital malformation in offspring's which contributes to neonatal mortality and morbidity. It occurs either before pregnancy (pregestational) or during pregnancy (gestational). Globally, the prevalence of diabetes in pregnancy in women is between ages 20-49 years [1]. The pregestational could be type 1 and 2 diabetes. Pregestational diabetes (PGD) possess high danger than gestation diabetes. In Nigeria, cases of diabetes during pregnancy is nearly 1.8%, from which 39% cases diabetes noticed before pregnancy and gestation-based diabetes is responsible for nearly 61% [2].

Occurrence of PGD is currently rising due to elevated cases of diabetes type 2 and obesity in women on child-bearing age [3]. It is among main causes of birth deformity, neonatal illness and death. Several researches revealed that cases of birth deformity linked to PGD is high compare to population of non-diabetic pregnancy[4-8]. PGD present danger of initial embryonic deformity because in most case it is not noticed or diagnosed early or before pregnancy [9,10] and massive proportion of pregnancy are not planned [11]. Growth retardation is one of the clinical conditions that results from pregnancy complicated with diabetes. Various factors have been shown to be responsible for this, some of which are genetic factor (variation of different maternal, foetal and placental genes) and foetal environment (water content, minerals, nitrogen and proteins, glycogen)[12-14]

In a worldwide survey, about 30 million infants suffer from intrauterine growth retardation yearly and the incidence is found to be higher in underdeveloped/developing countries than developed countries. It is also found to differ among populations, countries, and races and increases with decreasing gestational age. The highest incidence is seen in this order in the following continents: Asian, Africa, and Latin America [14,15]. The prevalence in Asian is reported to be 16.5% while in Nigeria is about 12.8% [16-18].

Plants have been used by human for medicinal purposes and as a basis for many pharmaceutical purposes. *Moringa oleifera* is one of those plants that have been used for medicinal and therapeutic purposes. It is an edible plant commonly known as (family: moringaceae) drumstick tree. It's widely grown in tropical and sub-tropical regions. In Nigeria it's commonly called moringa (English), okwe oyibo (Igbo), ewe igbale (Yoruba), Zogale (Hausa). All part of its plant has medicinal properties which have been used for the treatment of various ailments and diseases[19-21].

Due to the increase in the incidence of diabetes among women of child bearing age and its associated complication in pregnancy especially in the developing

foetus, there is urgent need of therapy that are effective and with no or less side effects. Hence this research was conceived so as to investigate the impact of *Moringa oleifera* extract on foetal growth parameters.

MATERIALS AND METHODS

Plant material and extraction: The leaves of *Moringa oleifera* was collected from various crop farms in Port Harcourt, Rivers state. The plant was identified and authenticated in the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State and deposited in the herbarium unit with an assigned voucher number UPH/P/103.

The leaves were air dried for 4 weeks (29°c-37°c), crushed and grinded into powder with electrical machine. The powdered leaves were extracted using ethanol solvent. The powdered leaves were weighed (680g) and Soxhlet extraction was conducted with ethanol (70%). The extract was transferred to water bath for solvent to evaporate and paste form of it was obtained and thereafter kept in tight bottle in refrigerator ready for use.

Experimental Animal: Thirty (30) femaleWistar rats, weighing 150g-200g were used for this study.They were purchased from the animal house of Pharmacology department, University of Port Harcourt, Rivers state, Nigeria. The animals were housed in cross-ventilated room (temperature 29±1.0°c, 12hr light /12hr dark circle) and fed with standard chow (feed) purchased from Top feeds (Premier feeds mills) and water ad libitum. They were allowed to acclimatize for two (2) week before the experiment commenced

Chemicals: Streptozotocin (STZ) (U-9889) sc-200719 was purchased from Santa Cruz Biotechnology 10410 Finnell Street. Dallas, TX 75220 and stored at -20°c. Insulin injection (Humulin 70/30) was obtained from E-blend Pharmacy located at university of Port Harcourt, Rivers State. It was manufactured by Eli Lilly and company, Indianapolis, IN46285, USA.

Induction of Experimental Diabetes: Diabetes was induced by a single dose of 45mg/kg body weight of streptozotocin (STZ) through intraperitoneal injection. The STZ was dissolved in sterile 0.1M sodium citrate with a buffer pH of 4.5 and injected within 10 minutes of preparation.Before STZ administration, these female animals were fasted overnight. The animals were confirmed diabetic after 72hours when the blood glucose level is higher than 11.1mmol/l.

Experimental Design: Following diabetic state of these animals in their respective groups (both non diabetic and diabetic) were mated with normal male Wistar rat.To confirm mating, vaginal smear was done in early hours of the morning to observe the presence of sperm cells with light microscope. The animals were then assigned to six (6) groups (n=5). Treatment commenced, plant extract was administered orally and insulin was given daily subcutaneously.

Group A: normal control rats, received water and feed;
Group B: diabetic rats (45mg/kg body weight STZ)
Group C: diabetic rats treated with 5IU/kg body weight/day insulin;
Group D: diabetic rats treated with 120mg/kg body weight *Moringa oleifera* extract;
Group E: diabetic rats treated with 240mg/kg body weight *Moringa oleifera* extract;
Group F: diabetic rats treated with 600mg/kg body weight *Moringa oleifera* extract.

After 20 days of treatment, the animals were dissected and the uterine horn were removed with the foetus, placed in a petri dish containing Phosphate buffer solution to separate foetus from surrounding decidua. The foetus was then transferred to clean petri dish for examination of external morphological deformity or abnormalities, number of implantation sites (using magnifying lens), and resorbed foetuses. The weight, crown rump length (CRL) and abdominal circumference of foetuses from all groups (5 litters each from each groups) was measured. The weight was measured using a digital weighing scale (g).

The CRL was measured using a digital ruler (mm). The measurement was taken as the maximum length from topmost point on the head to the most downward curved point on the tail (C-shaped).

The abdominal circumference was measured from an imaginary horizontal line at the midway region between the lowest rib margin and the iliac crest.

Statistical Analysis: Data were analysed with SPSS version 20.0 window and results obtained expressed as mean± SEM. All statistical comparisons were done using ANOVA followed by Tukey's post-hoc test. The difference showing P level of 0.05 or lower were considered statistically significant.

RESULTS

The study showed a significant decrease in the foetal weight, crown-rump length and abdominal circumference of the diabetic group compare to the control, insulin and *Moringa oleifera* treated groups (Table 1).

There was a significant increase in the foetal weight, crown-rump length and abdominal circumference of *Moringa oleifera* treated groups and insulin group compared to the diabetic group (Table 1)

Table 1: Effect of ethanolic extract of *Moringa oleifera* leaves on foetal weight, crown rump length and foetal abdominal circumference**.**

Groups	Foetal weight (g)	Crown-rump length (mm)	Abdominal circumference (cm)
A	3.27±0.06	31.64±0.72	3.59±0.07[b]
B	2.60±0.24[c]	28.40±0.40[c]	1.97±0.15[a, c]
C	3.59±0.17[b]	34.82±0.83[b]	3.21±0.18[b]
D	3.23±0.11	33.03±0.73[b]	3.37±0.13[b]
E	3.45±0.11[b]	34.27±0.88[b]	3.66±0.11[b]
F	3.81±0.08[b]	35.41±0.91[a, b]	3.63±0.12[b]

Values are given as mean ± SEM for each group. Superscript 'a', 'b' 'c 'indicate significant difference (at p˂0.05) compared to general control, diabetic and insulin respectively.

DISCUSSION

The growth and development of foetus during pregnancy is a complex process largely dependent on maternal condition (either nutrition or disease/infection) and the placenta, responsible for the supply of blood and nutrients to the foetus. In this study, there was reduction in the growth of the offspring of diabetic animals when compared to the control, insulin treated group and *Moringa oleifera* treated groups. This could be as a result of the diabetic condition of the mothers. Reports have shown that the penetration of maternal blood to the foetus during pregnancy exposes the foetus to toxic materials, especially under diabetes condition [22]. Also, insufficient transfer of nutrient and blood flow to the foetus is associated with foetal growth retardation. [23-25]. Studies have shown that growth retardation can be due to placental vascular insufficiency which deprive the foetus from necessary nutrition[26,27]. In a study by Rudge *et al*. [28], lower foetal weight was observed in the diabetic rats. They suggested that this may be due to insufficient passage of nutrients to the foetus because of the thickness of the placental membrane. This could be one of the factors that contributed to the decrease in foetal weight, crown rump length and abdominal circumference observed in the diabetic foetus as compared to the control and the *Moringa oleifera* extract treated groups in this study. More so, the growth retardation observed in this study may be due to the effect of maternal diabetes on foetal pancreatic beta cells because it was demonstrated at the later stage of foetal development in this study. At the later stage of development, the pancreatic islet cells become functional in the embryo from day 17/18 of gestation[29]. This implies that an effect on foetal pancreatic islet later in diabetic pregnancy may contribute to foetal growth retardation. This could also indicate that the exposure of embryo to diabetic state can predispose them to growth retardation. This is also reported by [30],[31],[32]. In this study foetal growth retardation was observed as shown by the lower mean weight, crown rump length and abdominal circumference. Other studies have reported the relationship of diabetes in pregnancy and macrosomia (larger than size/weight for gestational age). Macrosomia is known as one of the complications associated with diabetic pregnancies due to excessive growth in late pregnancy[33,34]. Macrosomia has been reported to be associated with gestational diabetes due to over expression of insulin and are more common in human diabetic pregnancy. The increase in foetal growth parameters noticed in *this work could be attributed to the nutrients present in Moringa oleifera extract. The extract has been found to be a good source of nutrients like vitamins, proteins, minerals, fat and carbohydrates* [35,36].

CONCLUSION

Diabetes in pregnancy is one of the major diseases that contributes to foetal anomalies. The decrease in the foetal parameters observed in this study arefoetal growth parameters are pointers to intrauterine growth retardation (IUGR). The study established that *Moringa oleifera* leaf extract can reverse the effect of maternal diabetes on the growth and development of foetus.

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