academicJournals

Vol. 10(4), pp. 26-32, April 2018
DOI: 10.5897/IJNM2018.0305
Article Number: AD59C5D56776
ISSN 2141-2456

http://www.academicjournals.org/IJNM

International Journal of Nursing and Midwifery

Full Length Research Paper

Pregnancy outcome and early postnatal weights in diabetic and non-diabetic pregnant rats administered ethanolic extract of *Ocimum gratissimum* leaves during pregnancy

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Received 1 March, 2018; Accepted 29 March, 2018

Extract of *Ocimum gratissimum* (OG), also known as scent leaf, is popularly used to treat diabetes mellitus and its hypoglycaemic activity has been confirmed by *in vivo* studies. The aim was to investigate the effect of this extract on placenta development and birth outcome in diabetic pregnancy. Forty two pregnant rats weighing 150-200 g were used. They were divided into control and extract treated diabetic and non-diabetic groups with 6 rats in each subgroup. Extract treated groups were administered 200 and 400 mg/kg of ethanolic extract of OG orally after the induction of diabetes by administration of alloxan monohydrate after an overnight fast. Treatment commenced in the second trimester and lasted till end of pregnancy. On day 18 of pregnancy, 3 rats from each group were sacrificed and placentae harvested and weighed. On the day of delivery, birth weight and other parameters were recorded. Results show that OG administration caused a significant decrease in placental weights, birth weights, litter sizes and placental-birth weight ratio in both diabetic and non-diabetic pregnancy. In conclusion, the observed decrease in placenta-birth weight ratios may suggest a protective beneficial effect of this extract against macrosomia in diabetic pregnancies and increased risk of cardiovascular disease later in life.

Key words: *Ocimum gratissimum*, gestational diabetes, placenta-birth weight ratio, pregnancy outcome, postnatal growth.

INTRODUCTION

There are evidences that diabetic intrauterine milieu is associated with adverse consequences for fetal and postnatal life. Gestational diabetes occurs when pregnant women without a previous history of diabetes develop a high blood glucose level (Casanova et al., 2005) which is characterized by an increased placental transport of

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glucose and other nutrients from the mother to the fetus, resulting in fetal macrosomia (kamana et al., 2015). However, in severe maternal diabetes complicated by vasculopathy and nephropathy, intra-uterine growth restriction (Dandrea et al., 2001), and in some cases seizures or stillbirth (Dave and Katyare, 2002) can be seen.

Maternal diabetes is also associated with concentration changes of various hormones, cytokines and metabolites in maternal as well as fetal circulation. Hence, these diabetic-associated changes are likely to affect the placenta, because receptors, transporters and enzymes, which are the primary targets of circulating molecules, are expressed often asymmetrically, on both placental surfaces (Desoye and Hauguel-de-Mouzon, 2007). Altered placental function in gestational diabetes may include changes in invasion, ultimately leading to an enhanced risk of early pregnancy loss, growth restriction and pre-eclampsia, as well as a long-term stimulatory effect on placental growth leading to placentomegaly, which is frequently associated with diabetic pregnancies (Emordi et al., 2016).

Gestational diabetes mellitus (GDM) poses a risk to mother and child. The two main risks GDM imposes on the baby are growth abnormalities and chemical imbalances after birth, which may require admission to a neonatal intensive care unit (kamana et al., 2015). Infants born to mothers with GDM are at risk of being both large-for-gestational-age (kamana et al., 2015) in unmanaged GDM, and small-for-gestational-age and intrauterine growth retardation in managed GDM (Godfrey, 2002). In addition, fetal macrosomia in turn increases the risk of instrumentation deliveries or problem during vaginal delivery (shoulder dystocia) (Haavaldsen et al., 2013). Neonates born from women with consistent high blood sugar levels are also at an increased risk of low blood glucose, jaundice, polycythemia, hypokalemia and hypomagnesemia (Irene et al., 2015). GDM also interferes with maturation, making immature babies prone to respiratory distress syndrome due to incomplete lung maturation and impaired surfactant synthesis (Irene et al., 2015).

Treatment of GDM is somehow problematic as care has to be taken to avoid low blood sugar levels due to excessive insulin injections. More injections can result in better control but requires more effort and there is no consensus as to its benefits (Karen et al., 2000; Jones, 2011; Haavaldsen et al., 2013) With the advent of modern obstetric care, the incidence of congenital malformations and neural tube defects have drastically reduced, but macrosomic babies and associated complications remain high (Kaufmann et al., 2003; Kelly et al., 2005).

Studies have shown that extract of OG has been used to treat diabetes mellitus and its hypoglycemic effect has been confirmed *in vivo* (Lee et al., 2005). Unlike most anti diabetic drugs, a study revealed that aqueous extract of OG leaf can significantly reduce postprandial hyperglycaemia in type-2 diabetic model rats, without the risk of hypoglycemia (Lumey, 1998). Most studies on its hyperglycemic effect have been carried out in males using animal subjects (Oguabobi et al., 2012; Shittu et al., 2016). Data on its hyperglycemic effect on diabetic pregnancies is scanty. Secondly, there is high rate of consumption of OG by pregnant women (either as spice or herbal drink) in some rural parts of Nigeria, with speculations that it reduces fetal weight thereby making delivery less laborious. This study was therefore undertaken to investigate whether OG, a confirmed anti-diabetic agent, would have any effect on placentomegaly and fetal macrosomia, which are usual complications of diabetic pregnancy.

MATERIALS AND METHODS

Plant preparation and extraction

Freshly matured leaves were purchased from Ekpoma market in Edo State, mid-west Nigeria and the leaves were identified and authenticated by Mr. Chijioke John Onyeukwu of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, where a voucher specimen (UNH Number 360b) was deposited. The leaves were air-dried in a dust-free environment and chopped into pieces using an electric blender. Ethanolic extraction of the leaves was carried out using soxhlet extractor as previously described (Metzger et al., 2008). The extract was then concentrated in a water bath and kept at -4°C until use. Phytochemical analysis of the leaf extract was determined using High Performance Liquid Chromatography method as previously described (Metzger et al., 2008).

Experimental animals

Forty-two matured nulliparous female rats weighing between 150-200 g were used for this study. The rats were housed in special clear-sided cages, with a 12:12 h light-dark cycle and were allowed free access to drinking water and standard rat pellet feed. They were allowed to acclimatize for two weeks.

The estrus cycle was monitored for each rat by daily examination of vaginal smears under the light microscope. At pro-estrus, male rats were introduced into the female cages to allow for mating. Mating was proven successful when spermatozoa were observed in the vaginal smear of the female rats the following morning and this was regarded as the first day of pregnancy.

Experimental design

After pregnancy was confirmed, rats were randomly divided into two broad groups; diabetic and non-diabetic groups. Each group had control and graded doses of extract treated sub-groups. Non-diabetic group consisted of control, 200 mg/kg b.w of extract, and 400 mg/Kg b.w of extract subgroups. Diabetic group consisted of control, 200 mg/Kg b.w of extract, 400 mg/Kg b.w of extract, and 0.5 IU/Kg b.w of insulin subgroups. Each of these seven subgroups had a total of eight rats.

Table 1. Phytochemical analysis of *Ocimum gratissimum* (Qualitative).

Test	Result
Ninhydrin	+
Xantheoprotic	+
Tannin	-
Glycoside	++
Terpernoid	++
Steroid	-
Flavonoid	+
Saponin	++
Resin	++
Alkaloid	++
Anthraqunine	-
Phenol	++

+ = slightly present; ++ = moderately present.

Table 2. Quantitative analysis of OG.

Constituent	% Composition
Alkaloids	2.5
Saponin	3.0
Glycoside	0.0155
Flavonoid	0.5205
Phenol	0.1547

Rats were fasted for 12-h before diabetes was induced on day eight of pregnancy using alloxan monohydrate dissolved in normal saline (Myatt, 2006). The injection site was swabbed using iodine solution. While the rat was held in a dorsal position, the amount of alloxan was injected into the caudal vein at the base of the tail using sterile 1ml syringe.

Blood was taken from the tail vein of all rats, and placed on ACCU-CHEK Active test strips, to check for blood glucose level, before induction of diabetes mellitus, using ACCU-CHEK Active glucometer, Roche Germany. This record was taken as the baseline value for blood glucose. The blood glucose level was subsequently checked 24 h after induction. Animals whose blood glucose exceeded 200 mg/dl were considered diabetic (Nahum et al., 1999).

After confirmation of diabetes, rats in the extract treated groups, both diabetic and non-diabetic, were given graded doses of 200 and 400 mg/kg body weight using oral dosing syringe at the second week of gestation. A subgroup of rats in the diabetic group was given 0.5 international units of soluble insulin per kg body weight and was administered using insulin needle intraperitoneally (Oguabobi et al., 2012). The non-treated control groups received feed and water only. The treatment lasted till parturition.

Body weight (using digital electronic weighing scale), and blood glucose levels were monitored daily, after induction, until delivery. This was to assess the severity of the induced diabetic state.

At day 18 of pregnancy, half the number of rats from each group was sacrificed by cervical dislocation according to Institutional Animal Care and Use Committee (IACUC) guideline on euthanasia (Silverman et al., 2014). The placentae and fetuses were harvested for determination of placental and fetal weights and placental-fetal weight ratio.

On the day of delivery, the litter sizes were noted, birth weight was measured and recorded to the nearest (mg) using an electronic weighing scale, and litters were examined for any anatomical malformations.

Ethical clearance on animal use and handling was obtained from College Of Medicine Research ethics committee, University of Nigeria. A copy of the ethical clearance was attached as an appendix.

Statistical analysis

Results were presented in tables as M±SED and compared using student t-test. Level of significance was taken as $p< 0.05$.

RESULTS

Phytochemical analysis of *O. gratissimum* (qualitative) is shown in Table 1. From the qualitative analysis of O.G, it was observed that saponin, alkaloid, phenol, resin, glycoside, terpernoid and carbohydarate, ninhydrin, xantheoprotic, and flavonoid were present in the extract with alkaloids and saponin relatively higher in concentration as observed in the quantitative analysis result (Table 2).

The result from Table 3 shows that the extract of *O.*

Table 3. Effect of consumption of ethanolic extract of *Ocimum gratissimum* on placental weight (g) at day 18 of pregnancy.

Treatment/Groups	Non-diabetic	Diabetic
Control	0.56±0.04	0.65±0.03
200mg/Kg B.W	0.38±0.01*	0.38±0.03*
400mg/Kg B.W	0.28±0.03*	0.31±0.04*
ITDP 0.5 IU/Kg B.W		0.36±0.01*

Values are expressed as mean ± SEM, n= 6. *Significantly different from the control at $p < 0.05$.

Table 4. Effect of consumption of ethanolic extract of *Ocimum gratissimum* on litter weight at birth.

Treatment/Groups	Non-diabetic	Diabetic
Control	4.44±0.15	5.92±0.27[a]
200mg/Kg B.W	4.75±0.40	4.24±0.40*
400mg/Kg B.W	4.77±0.20	4.48±0.16*
ITDP 0.5 IU/Kg B.W		5.41±0.36

Values are expressed as mean ± SEM, n= 6. *Significantly different from the control in same group at $p < 0.05$. [a]Significantly different between group at $p < 0.05$.

Table 5. Effect of consumption of ethanolic extract of O*cimum gratissimum* on litter size at birth.

Treatment/Groups	Non-diabetic	Diabetic
Control	6.67±0.33	7.00±0.58
200mg/Kg B.W	4.33±0.33*	4.33±0.33*
400mg/Kg B.W	4.67±0.88	6.00±1.00[a]
ITDP 0.5 IU/Kg B.W		5.5±0.50

Values are expressed as mean ± SEM, n= 6. *Significantly different from the control in same group at $p < 0.05$. [a]Significantly different between group at $p < 0.05$.

gratissimum significantly reduced the placental weight in a dose-dependent manner when compared with the control which did not receive the extract. There was no significant difference in the effect of the extract on placental weight between the diabetic and non-diabetic group.

There was no significant reduction in litter weight in the extract treated non-diabetic group compared with the control as shown in Table 4. However, the extract appeared to cause a significant reduction in litter weight in diabetic group when compared with the control and insulin group as shown. In addition, control diabetic group had a significantly higher litter weight when compared to control in non-diabetic group.

The result (Table 5) shows that the 200 mg extract treated group had lesser litter size compared with the control, whereas the 400 mg group was not significantly different from the control and 200 mg group in the non-diabetic group (Table 5). The 200 mg extract treated group had a significantly number of litter size than the 400 mg, ITDP 0.5 IU/kg/bw and control in the diabetic groups. However, litter size was significantly higher in 400 mg/kg/bw in diabetic group when compared to the non-diabetic group as shown in Table 5.

It was observed that the extract reduced the placental-birth weight ratio when compared with the control as shown in Table 6. In the diabetic group, insulin and extract (400 mg/kg/bw) showed a significant decrease in the placental-birth weight ratio when compared with the control. However, on comparison of non-diabetic and diabetic groups, the diabetic groups had significantly greater placental-birth weight ratio as shown below.

The Figure 1 shows a significant increase in weight of the 200 mg extract treated group when compared with the control throughout the three weeks. The 400 mg extract treated group all died two days postpartum.

From Figure 2, there was a steady increase in the weight of the offspring in both the 400 mg treated group

Table 6. Effect of consumption of ethanolic extract of *Ocimum gratissimum* on placental-birth weight ratio.

Treatment/Groups	Non-diabetic	Diabetic
Control	0.09±0.00	0.15±0.01[a]
200mg/Kg B.W	0.06±0.00*	0.08±0.01[a]
400mg/Kg B.W	0.06±0.01*	0.07±0.00*
ITDP 0.5 IU/Kg B.W		0.06±0.01*

Values are expressed as mean ± SEM, n= 6. *Significantly different from the control in same group at $p < 0.05$. [a]Significantly different between group at $p < 0.05$.

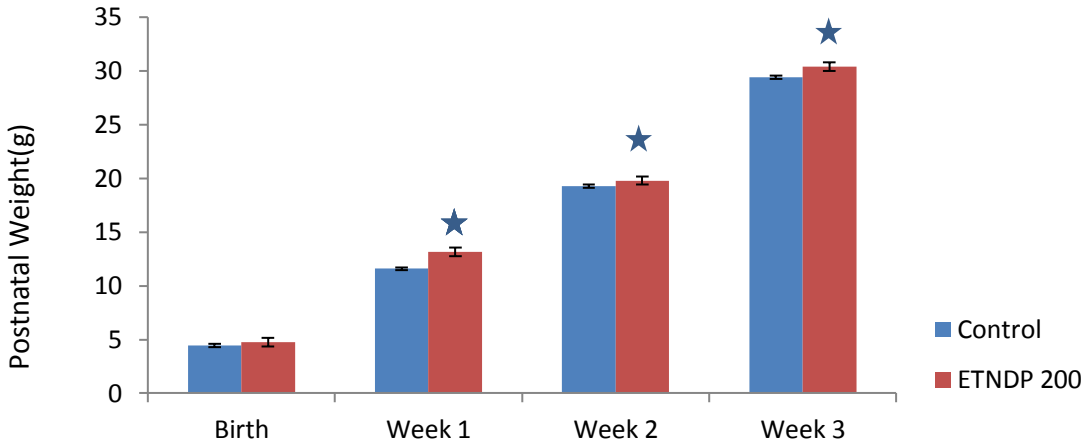

Figure 1. Effect of consumption of ethanolic extract of *Ocimum gratissimum* by non-diabetic pregnant *rats* on postnatal weight of offspring. ETNDP 200 = extract treated non-diabetic pregnant, 200 mg/Kg b. wt; ETNDP 400: all died two days postpartum. N= P<0.05 compared with control.

and the insulin group. The control group and 200 mg treated group all died before the end of the first week postpartum.

DISCUSSION

The observed decrease in placenta weight in both the diabetic and non-diabetic group in the present study, which appeared to be concentration dependent, may be due to the presence of phenol. Phenol was shown to be an active component of the extract (Tables 1 and 2). Omodamiro et al. (2012) has previously reported the presence of phenol in this extract. Lee et al. (2005) showed that phenol decrease placenta weight by significantly reducing levels of placenta lactogen, prolactin-like protein A and C, and decidual prolactin related protein. Placenta lactogen, which is released by a term placenta, affects glucose and insulin metabolism, by decreasing maternal glucose utilization which helps ensure adequate fetal nutrition (Omodamiro et al., 2012). It also decreases maternal insulin sensitivity leading to an increase in maternal blood glucose levels (Rich-Edwards et al., 1997). Consequently, decreasing levels of this anabolic hormone will go a long way in reducing placental weight and by extension, fetal weight. Though prolactin-growth hormones were not quantified in this study, however, the result is in line with previous study by Lee et al. (2005) who reported that bisphenol A, an estrogen-like environmental endocrine disrupter, reduced the secretion of the placental prolactin-growth hormone leading to reduced placental and fetal weight and litter size.

There was a significant increase in the resorption sites following extract administration. This suggests that the extract may have interfered with the normal processes of implantation. This observation may be due at least in part to the presence of alkaloid in the extract (Table 2). Alkaloid administered orally in the second trimester has been reported to cause anti-implantation, anti-gonadotropic, anti-progesteronic, selective estrogenic, embryonic resorption and feto-toxic activities without inducing abortions in animals (Yakubu and Musa, 2012). Ting et al. (2014) reported that Dipsaci Radix (which is

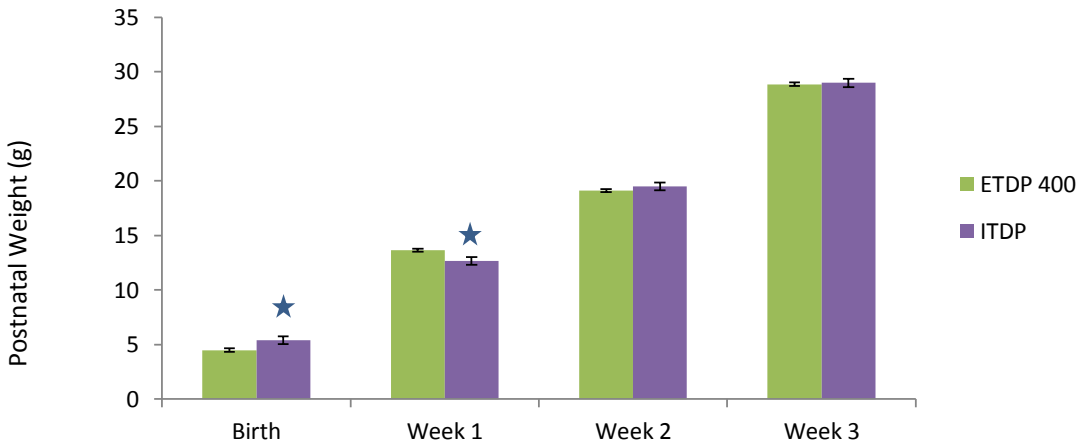

Figure 2. Effect of consumption of ethanolic extract of *Ocimum gratissimum* by diabetic pregnant rats on postnatal weight of offspring. ITDP = Insulin treated diabetic pregnant. ETDP 400 = extract treated diabetic prgnant, 400mg/Kg b. wt; ETDP 200 and Control diabetic: all died within 7 days postpartum; N = P<0.05 compared with control.

high in alkaloid) at a high-dose and long-term administration led to adverse impacts in maternal health and embryo-fetal development in mice and embryonic stem cells. Our results is in support of these previous findings as increased resorption sites may have led to a decrease in the number of viable fetuses that ultimately led to decreased litter size observed in our study.

Adequate placental function is necessary for delivery of nutrients, oxygen, and hormones to the fetus (Setji et al., 2005). The placental-birth weight ratio could be a useful marker for placental efficiency and efficacy (Risnes et al., 2009). Thus, a comparatively large placenta relative to birth weight may be an expression of a relatively inefficient placenta with reduced ability to translate its own growth into fetal growth (Risnes et al., 2009). Studies suggest that a placenta that is large relative to birth weight may be an indicator for reduced nutrient supply to the fetus (Thomas, 2005; Stephanie et al., 2014). In this study, it was observed that extract administration decreased the placental-birth weight ratio possibly in a dose-dependent manner. Both small and large placentae relative to birth weight have been reported to be associated with death in preterm births (Ting et al., 2014). Studies on developmental programming have reported that increased placental-birth weight ratio increases the risk of cardiovascular disease later in life (Van-Assche et al., 1998; Walkinshaw, 2006; WHO, 2014). This observed decrease in the placental-birth weight reported in our study may have been as a result of the extract-induced decrease in the placenta weight reportedly caused by the phenol constituent of the extract.

As shown in Figure 1, it was observed that low dose of OG accelerated early postnatal weight of offspring in non-diabetic pregnancy while Figure 2 shows that high dose extract had similar effect as insulin on early postnatal weight of the offspring in diabetic rat. During the postnatal phase, the litters feed on the breast milk of their mothers. The accelerated growth observed may be from the composition of the breast milk. Since OG is a galactagogue (Yakubu and Musa, 2012), it may have enhanced breast milk production as well as its composition. OG was observed to be toxic at high dose for non-diabetic offspring as there was 100% neonatal mortality. Though low dose extract was not protective against the complications of diabetic pregnancy as both mother and offspring died during the early postnatal period, high dose extract was however protective against the complications of diabetes in the diabetic group.

Limitation to this study

A major limitation to this study is the failure to ascertain the lethal dose of this extract through acute toxicity test. Further studies are recommended to determine the suitable dose of the extract to eliminate fetal resorption associated with the use of this extract as reported in this study.

The research work was done during the raining season between the months of June and July which is usually characterized by low temperature. Failure to maintain a constant room temperature may have contributed to the increase in mortality rate of the offsprings.

Conclusion

In conclusion, these results have shown that ethanolic

extract of *O. gratissimum* has the potentials to reduce placental weight and birth weight, decrease litter size and accelerate early postnatal growth of offspring in both normal and diabetic conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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