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Original Article

Assessment of *Bridelia ferruginea* benth for its therapeutic potential in pregnancy-induced impaired glucose tolerance in rats

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ABSTRACT

Background: Pregnancy induced diabetes also known as gestational diabetes develops during pregnancy. Pregnancy is an insulin resistant state that may induce impaired glucose tolerance and often gestational diabetes in susceptible women. Gestational diabetes causes serious problems to the mother and the baby. Therefore, the use of herbal remedies such as *Bridelia ferruginea* with the potential ability to improve glucose tolerance during pregnancy will definitely improve pregnancy outcome in gestational diabetes. **Aim**: This paper presents an evaluation of possible therapeutic potential of *B. ferruginea* in gestational diabetes by assessing the anti-glycaemic effects of the plant's aqueous extracts on pregnancy-induced glucose intolerance in rats. **Materials and Methods:** Adult virgin, timed-pregnant and non-pregnant rats were subjected to brief ether anaesthesia following 18-hour overnight fasting period to allow for oro-gastric administration of glucose load at 3.0g/kg body weight as 30% solution. The oral glucose load was given at an administration volume of 1.0ml/100g bw. **Results:** Oral glucose tolerance test showed that pregnancy induced glucose intolerance in the rats. However, *B. ferruginea* caused a reduction in glycaemic response to glucose challenge and an increased glucose tolerance in rats that had pregnancy-induced glucose intolerance. Thus, diabetogenic effect of pregnancy was ameliorated by oral administration of aqueous extracts of *B. ferruginea* to pregnant albino rats.

Key words: Gestational diabetes, pregnancy-induced diabetes, hypoglycaemic, glycaemic response, glucose tolerance.

INTRODUCTION

In many developed nations of the world, diabetes had been a source of concern in view of its associated morbidity and mortality. According to WHO as reported by Bnouham *et al*.[1] about 150 million people worldwide has diabetes mellitus, and this number may be double by 2025. The rising incidence is of particular interest in developing countries because the problem of diabetes is compounded by poverty and ignorance. Generally, diabetes can be classified into two broad categories namely type 1 and type-2 diabetes.[2] Pregnancy induced diabetes or gestational diabetes is another important class of diabetes that has not received comparable level of attention, especially in the developing countries, despite its severity and increasing prevalence. It is characterized by carbohydrate intolerance of variable intensity with onset or first recognition during pregnancy.[3]

Pregnancy is an insulin resistant state which is often characterized by impaired glucose tolerance in susceptible pregnant women. A proportion of women with pregnancy-induced impaired glucose tolerance may become diabetic. Thus, occurrence of impaired glucose tolerance needs to be well managed in order to prevent or, at least, delay onset of gestational diabetes. In many developing countries, many preventable fetal and neonatal deaths are associated with diabetes-related pregnancy. Babies born of diabetic pregnant mothers are macrosomic (large), and unhealthy resulting in higher perinatal complications.[3] Also, in many developed countries, the frequency of gestational diabetes is increasing. The long-term implications for developing countries like Nigeria are of serious concern. Many cases of diabetes associated with pregnancy are preventable if adequate attention is paid to pregnancy-induced impaired glucose intolerance which might pre-exist before frank diabetes. In view of the increasing prevalence of gestational diabetes and the associated risks of maternal and neonatal morbidity and mortality, this class of diabetes remains a significant challenge and increasing attention should be focused on its management especially during the pre-diabetic phase of impaired glucose tolerance.

The management of pregnancy-induced diabetes is generally by the administration of hypoglycaemic drugs. In some parts of the world especially in poor developing countries like Nigeria, the success of conventional diabetic treatment has raised some concern because of the associated problems of side effects, non-compliance with recommended drug regimes, and prohibitive costs of conventional drugs. In recent times, there has been a resurgence of interest in herbal medicine in many parts of the world in view of the acclaimed therapeutic efficacy of many herbal preparations.[4] It is generally believed that herbal medicine does not only agree with cultural and socio-economic peculiarities of many developing countries, it also symbolizes safety in contrast to conventional drugs. In view of this, herbal medicine is becoming a focus for a wider coverage of primary health care delivery in Africa and, possibly, the rest of the world.

Bridelia ferruginea Benth (Euphorbiaceae) is a common medicinal plant that has been widely reported for its pharmacodynamic effects especially its antidiabetic properties. Long ago, Iwu[5] had demonstrated its hypoglycaemic effect in alloxan-induced diabetic rats. More recently, Kolawole *et al*.[4] showed that *B. ferruginea* reduced plasma glucose in normoglycaemic and glucose-induced hyperglycaemic rats. An extensive search on the literature revealed little or no information on the possible therapeutic effects of this plant on gestational diabetes. The present study was therefore carried out to see possible effect of *B. ferruginea* on pregnancy-induced impaired glucose tolerance in rats with a view to assessing its potential as an herbal remedy for human gestational diabetes.

MATERIALS AND METHODS

Experimental animals

Sprague-Dawley albino rats of both sexes weighing 160-180g were obtained from the Laboratory Animal Unit of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, and transferred to the animal room of the Biological Garden, University of Lagos, where they were kept in plastic cages for acclimatization for 3 weeks and subsequent mass breeding for 6 months to increase the stock. The cages were thoroughly cleaned and the animals examined on a daily basis. Clean tap water and rat feed were made available *ad libitum*. The temperature of the animal room was $30\pm3^{o}C$ with 12h:12h light-darkness cycle. On this regime, the animals were uniformly healthy and active. Rats that became pregnant were separated into solid floor maternity cages during the antenatal and the postnatal periods. Fine sterilized wood shavings were provided in the cages as bedding and nesting materials for the pregnant animals. Immediately after the offspring were weaned (i.e. before attaining sexual maturity), they were transferred into new cages where they were kept separately as only females or males to avoid mating. Thus, the animals remained virgin males and females before induction of pregnancy. Strict adherence of experimental procedures to ethics in animal experimentation was ensured throughout the study.

Collection and extraction of plants

The plants were collected from the wild around the Biological Garden of the University of Lagos, Akoka, Lagos, Nigeria. They were authenticated as *B. ferruginea* at the Botany Department, University of Lagos, Lagos. Nigeria. The leaves were removed, washed free of sand, cut into pieces, air-dried and ground into powder. Fifty grams of the powder was extracted with 250 ml of distilled water using the well known Soxhlet method. The extract was slowly evaporated *in vacuo* to obtain a total yield of 2.7g. Weighed sample of the dried extract

was used to prepare solution of extract for oral glucose tolerance test (OGTT).

Induction of pregnancy

After 90 days of life, 30 virgin rats (15 females and 15 males) obtained from mass breeding above were housed in 15 mating groups of monogamous pairs (1 female: 1 male per cage). At this period of life, the animals had attained sexual maturity and the vagina had opened. To check for successful mating, the animals were examined every morning to obtain vagina smears for microscopic observation of sperm cells. In addition, the vagina and the floor of the cages were checked for the presence of cornified plugs. The presence of sperm cells in the vagina smear or the availability of cornified plug in the vagina or on the floor of the cage indicated successful mating, and this was regarded as Day-1 of gestation. Such females were separated into maternity cages to constitute pregnant rats for OGTT which was carried out on Day-17 of gestation.

Oral glucose tolerance determination

Since successful mating does not imply successful pregnancy, the criterion for inclusion of animals in the pregnant group was confirmation of pregnancy which was usually physically obvious on Day-17 of gestation in view of the significant protrusion of the abdominal region due to the presence of well developed fetuses in the uterus. Female rats (n=15) consisting of 10 pregnant rats and 5 non-pregnant rats were used for OGTT on Day-17 as described in previous reports.[[6]] Essentially, the animals were fasted for 18 hours before the test, and a glucose load of 3.0/kg body weight (bw) was delivered into the stomach through the buccal cavity as 30% solution by oro-gastric intubation under light ether anaesthesia. Using an automated digital blood glucose analyzer, glucometer (Accu-Chek Advantage, Roche, USA), Blood glucose was obtained from cut tail tips for determination of blood glucose concentration just before oral glucose loading (0 minute) and at 30, 60, and 120 minutes of OGTT to obtain the glucose tolerance curve of each rat.

Experimental design and administration of materials

Three groups of female animals were used as follow: The pregnant diabetic rats were randomly divided into two groups of 5 animals each; one group received extracts of *B. ferruginea* (Pregnant Diabetic Treatment) while the other did not receive the extract (Pregnant Diabetic Control). The third group was made up of non-pregnant females that did not receive plant extract (Normal Control; n= 5). For OGTT in the pregnant diabetic treatment group, the plant was administered as glucose-extract solution constituted such that the dose of the plant extract was 250.0 mg/kg while the glucose load was 3.0 g/kg in an administration volume of 1.0ml/100gbw. The dose was established from a preliminary dose-effect study (data not presented) which indicated 250.0mg/kg as the minimum but significantly effective dose. The other two control groups (Pregnant Diabetic and Normal) were given only 30% glucose solution for the test. Blood samples were obtained from the tail for the determination of blood glucose concentration just before oral infusion of glucose as the only solution or as a solution of glucose combined with the extract (0 minute) and subsequently at 30, 60, and 120 minutes for OGTT.

Data analysis

Descriptive statistics and other statistical analysis were done using IBM SPSS Version 19 statistical software package. Results of blood glucose determinations are given as mean$\pm$SEM. Differences between means were determined by unpaired Student's t-test and $p<0.05$ was considered significant. When comparison of means involved more than two groups, one-way analysis of variance (ANOVA) was done followed by Turkey's post hoc Test. Association between parameters was assessed from scatter plots and correlation analysis. The glycaemic response for each rat was assessed by glycaemic response index (GRI), and it was obtained by determining the incremental area under its glucose tolerance curve. This was calculated by summation of the areas of trapezoids defined by individual points on the curve.[[7]] Since the glycaemic response was found to be indirectly related to glucose tolerance, a direct assessment of blood glucose handling, GTI or glucose tolerance index, was also determined by taking the reciprocal of (GRI).

RESULTS

Effect of pregnancy on glucose tolerance

By comparing the pattern of plasma glucose profiles of the pregnant and the non-pregnant rats, it was observed that pregnancy induced glucose intolerance in the rats (Fig. 1). It could be observed that the starting blood glucose concentration of the pregnant rats was 6.9$\pm$0.4, a value not significantly different from 7.0$\pm$0.7 mmol/l, the starting blood glucose concentration of the non-pregnant animals.

After 90 minutes of OGTT, the blood glucose concentration reached a peak level in both groups. However, the peak plasma glucose concentration in the pregnant rats (11.0±0.7 mmol/l) was significantly higher (P<0.05) than that of the non-pregnant rats (9.5±0.8 mmol/l). After 120 minutes, the blood glucose level of the non-pregnant rats dropped to 7.2±0.6 mmol/l, a value which was near the starting level; however, the corresponding blood glucose value in the pregnant rats remained significantly higher (8.8±0.7; P<0.05). Determination of glycaemic response index (GRI) and glucose tolerance index (GTI) showed that pregnancy caused a very significantly higher GRI and lower GTI in the rats (Table 1).

Glycaemic effect of *Bridelia ferruginea* on pregnancy-induced impaired glucose tolerance in the rats

The fasting blood glucose concentration of pregnant rats administered with *B. ferruginea* was 7.0± 0.7 mmol/l, a value that was comparable to the starting BGC of those that did not receive the extract (6.9± 0.8 mmol/l; P>0.05). After 90 minutes of OGTT, the blood glucose concentration reached a peak level of 11.0±0.7 mmol/l in the untreated pregnant rats while a lower peak value of 10.6±0.7 mmol/l was attained in the treated pregnant rats. At 120-minute time point, the blood glucose level of the pregnant rats dropped to 8.8±0.7 mmol/l, a value higher (P<0.05) than that of the normal control (7.2±0.6 mmol/l). Determination of glycaemic response index (GRI) showed that the pregnant animals treated with plant extracts had higher GRI which was 210.5.0±35.0 mmol.min/l when compared to the normal control group with mean GRI of 170.0±25.0 mmol.min/l but significantly lower (P<0.05) when compared to the GRI of the pregnant animals that were not treated with the extract (270.0±50.4 mmol.min/l). As assessed by glucose tolerance index (GTI), pregnant animals that did not receive *B. ferruginea* had the lowest glucose tolerance (Table 1). As can be observed from correlation values and the considerable scatter of data points in Fig. 2, glucose tolerance was not associated with body weight in the animals.

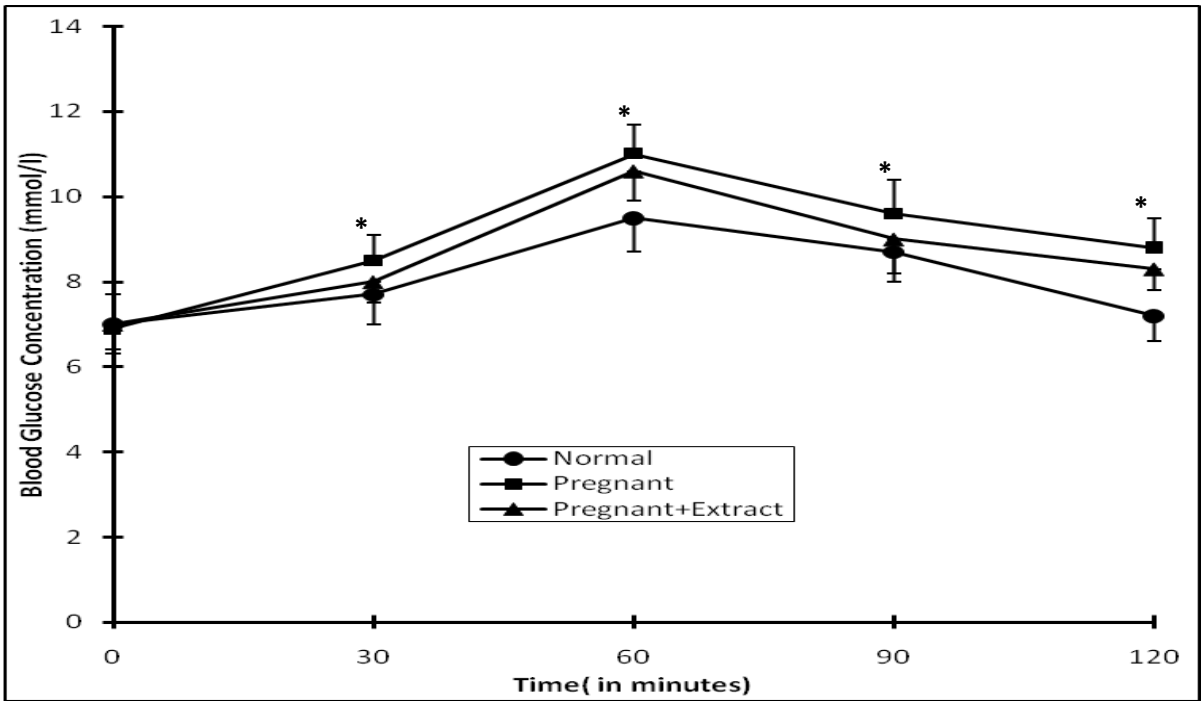

Fig. 1: Plasma glucose profiles in different groups of rats showing the glycaemic effect of B*ridelia ferruginea* in pregnancy induced glucose intolerance. *Significant difference from normal at *p*<0.05

Table 1: The effect of B*ridelia ferruginea* on glycaemic response and glucose tolerance in pregnant albino rats.

	Glycaemic Response Index (GRI) mmol.min/l	Glucose Tolerance Index (GTI) $mmol^{-1}.min^{-1}.l$
Normal	170.0±20.5	$5.9±0.3 \times 10^{-3}$
Pregnant	270.0±50.4*	$3.7±.07 \times 10^{-3}$*
Pregnant+ *Bridelia*	210.5±35.0	$4.8±0.9 \times 10^{-3}$

*Significant difference from normal at $p<0.05$

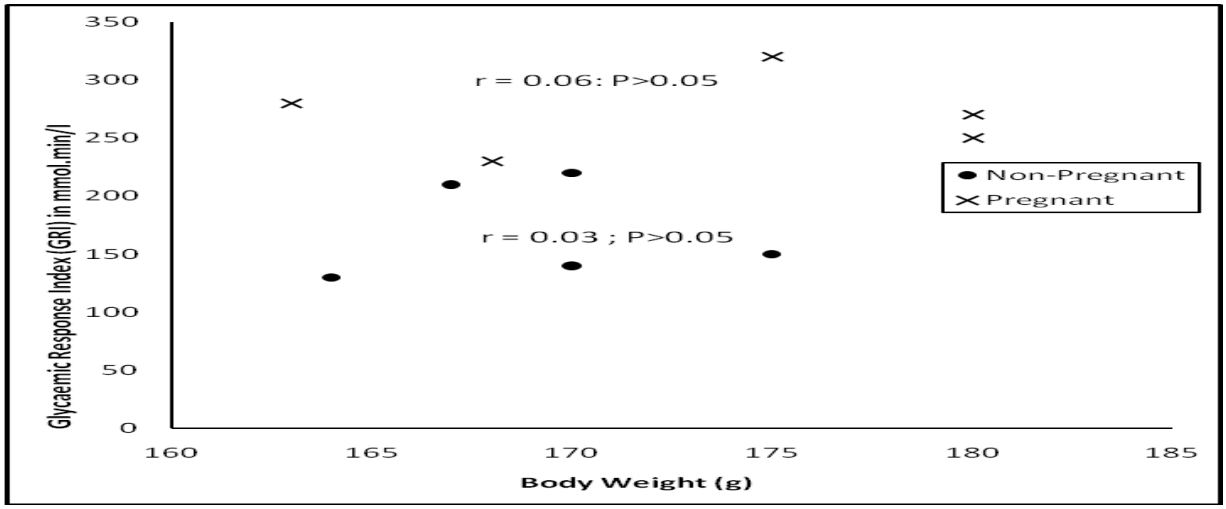

Figure 2: Lack of association between body weight and glycaemic response in normal and pregnant rats. Note: The body weight in the pregnant rats was the body weight before pregnancy.

DISCUSSION

The glucose tolerance curves of the pregnant rats (treated and non-treated) are clearly above that of the normal rats suggesting that pregnancy induced glucose intolerance in the rats. In our judgment, this deviation was not significant enough to qualify the pregnant rats as diabetic. However, the relative location of the curves with respect to that of the non-pregnant rats suggested that pregnancy has induced impaired glucose tolerance in the rats. This was attested to by the higher glycaemic response values and the corresponding lower mean glucose tolerance value of the diabetic rats as compared to their non-diabetic counterparts.

This observation had been corroborated by several human and animal experimental studies that pregnancy is an insulin-resistant state. In the light of this well known effect of pregnancy on carbohydrate metabolism, the higher glycaemic response and the impaired glucose tolerance in the pregnant rats used in this study could therefore be attributed to the well known diabetogenic effect of pregnancy.[11,12,13] Noting that gestational period in rats is about 22 days, using 17-day pregnant rats as was done in this study was intended to simulate late gestation period (3rd trimester) in human when gestational diabetic women usually have increased fasting insulin concentration, reduced glucose utilization, and less suppression of hepatic glucose production.[8] Unfortunately, Insulin assay could not be done in this study; concurrent measurement of

insulin with blood glucose during OGTT would have shed more light on whether impaired glucose tolerance due to pregnancy was associated with hyperinsulinemia in these rats. Many workers including Dowse[9] and Adebisi *et al*.[10] have demonstrated that obesity is positively associated with insulin sensitivity. Since the animals used in this study were non-obese, the lack of association between body weight and glucose tolerance in the present study corroborated the fact that body weight within the normal range is not related to glucose intolerance or insulin resistance.

The data obtained in the present study showed that pregnancy may induce impaired glucose tolerance in albino rats. Previous studies relating to animal models of gestational diabetes involved administration of streptozotocin, STZ,[11] or fructose[12] before or during pregnancy. However the adequacy of these models in reproducing human gestational diabetes has been questioned[13] partly due to the fact that these agents (STZ and fructose) are themselves diabetogenic[14,15] thus, the diabetogenic effect of pregnancy is complicated by the effect of these agents. Hence, induction of diabetes in animals by pregnancy without administering other diabetogenic agents as was done in this study may represent more appropriate animal models of human gestational diabetes.

It is generally believed that complications of gestational diabetes are usually manageable and preventable if blood sugar level is carefully controlled just as soon as the diagnosis of diabetes is made. Importance of proper diagnosis has been emphasized because pregnancy outcome in gestational diabetes is usually poor particularly in undiagnosed cases. Moreover, prenatal and perinatal complications are often high for the mother and especially the baby as a result of life-threatening complications like macrosomia, birth injury, and respiratory distress.[20] It was observed in the present study that extracts from *B. ferruginea* decreased glycaemic response to glucose challenge in pregnant rats with impaired glucose tolerance. Several years ago, Iwu[5] reported hypoglycaemic effect of *B. ferruginea* in non-pregnant patients receiving treatment from a local healer whereby the plant extracts in daily doses effectively reduced hyperglycaemia of diabetes. A more recent study by Kolawole *et al*.[4] had corroborated Iwu's report. The present data provides additional information that *B. ferruginea* reduced hyperglycaemia in pregnancy-induced impaired glucose tolerance in rats. Considering impaired glucose tolerance as a pre-diabetic state in certain diabetic cases and the severity of pregnancy-induced diabetes, the results of this study are important in the light of its implications for the management of human gestational diabetes. This is especially so in poor developing countries like Nigeria where problems of high cost of conventional drugs, non-compliance with therapeutic regimes, and various undesirable side effects are associated with conventional gestational diabetic treatment.

Several mechanisms could account for the glycaemic effect of *B. ferruginea*. Hypoglycaemic agents could exert their effects at several points of carbohydrate metabolism including digestion and absorption of carbohydrates, hepatic glycogenesis, increased insulin secretion by the beta cells and receptor-mediated glucose uptake for metabolism by the peripheral tissues. The weight of evidence supports possible role of *B. ferruginea* in the secretion or activation of endogenous insulin because the extract is more active in animals with healthy beta cells than in rats with damaged beta cell due to by pancreatic beta cytotoxic agents like streptozotocin[11] or alloxan.[21] Thus, *B. ferruginea* may not be effective in the treatment of type-1 diabetes which is characterized by insulin lack due to little residual or complete absence beta cell mass. It may however be effective in the treatment of type-2 or pregnancy induced diabetes where the pathophysiology is not that of insulin lack but insufficiency of insulin secretion to compensate for insulin resistance caused by pregnancy or other conditions such as obesity or other factors which may be genetic or at least have genetic factors as a component in their aetiology. Reports from previous phytochemical studies had associated the anti-diabetic properties of *B. ferruginea* to the presence of tannins, polyphenols, steroids, triterpenes and alkaloids.[4,5]

Gestational diabetes is conventionally managed by oral hypoglycaemic drugs. According to Smithberg and Runner[18] and Smoak[19] as reported by Ho,[20] there are major concerns regarding the use of oral hypoglycaemic drugs in pregnancy in view of the associated foetal anomalies, neonatal hypoglycaemia and the development of pre-eclampsia. The current view implies caution in the use of oral hypoglycaemic agents in pregnancy, and the benefit and costs of giving these drugs should be considered. A recent study by Kolawole and Sunmonu[21] on the effect of wastewater treated with methanolic extract of *B. ferruginea* is pertinent. The importance of herbs such as *B. ferruginea* in healthcare delivery cannot be overemphasized in a

resource poor country like Nigeria where the majority of people live in rural areas, and compliance with conventional drug therapy may be poor.

The results of this short-term animal experimental study suggest that *B. ferruginea* may play a beneficial role in the management of pregnancy induced diabetes in human. However, there is need for caution in directly extrapolating the outcome of animal experimental studies to man. More animal experimental and human studies are therefore needed in order to ascertain the beneficial effect of *V. amygdalina* in human gestational diabetes.

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Conflict of Interest: None declared