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Effects of Aqueous Extract of Bitter Kola (*Garcinia kola*) on the Pregnancy Outcome and Early Postnatal Development of the Offsprings of Diabetic Pregnant Rats

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ABSTRACT
Bitter *kola* (*Garcinia kola*) is a medicinal plant with a wide range of pharmacological effects including antidiabetic and antioxidant effects. In this study, the effects of aqueous extracts of *Garcinia kola* seed on the pregnancy outcome and early postnatal development of the offspring of pregnant diabetic and non-diabetic rats was studied. Forty (40) female rats were used. Pregnancy was induced in all the rats, and diabetes induced in twenty (20) making two groups; pregnant diabetic and pregnant non-diabetic. These two groups were further subdivided into four groups of five rats each receiving different concentrations of the extract as follows; control, 100mg, 200mg, and 300mg/kg of body weight. The extract was administered orally as a single dose daily throughout gestation. The extract caused a reversal of the significant reduction of weight gain and significantly increased weight gain among the pregnant diabetic rats. It also significantly reduced the fasting blood glucose concentration in the hyperglycemic diabetic rats in a dose-dependent manner to values close to normal. These may be due to the insulinogenic effect of kolaviron an active principle of Bitter kola The extract significantly increased the litter size among the diabetic pregnant rats in a dose-dependent manner when compared with their control that showed a statistically significant reduction in litter size. This observed effect of the extract may be because of the anti-oxidative stress effects of kolaviron and ascorbic acid (constituents of Bitter kola) observed in previous studies. The extract also reduced the birth weight, excessive early post natal growth, and the high fasting blood glucose concentration on the weaning (21st) day, among the offspring of diabetic rat in a dose-dependent manner when compared with those of the diabetic pregnant control group. These may be due to the blood glucose lowering effect of the aqueous extract of Bitter kola seed among their mothers which leaves little or no excess glucose for the fetus to absorb and as such avert the major complications of diabetic pregnancy. The result of this study suggests that Bitter kola may have a protective effect against the adverse effects of diabetes in pregnancy on both the mother and the offspring.

Keywords: Acute toxicity, phytotherapy, diabetes mellitus, animals, pregnancy, birth defects

INTRODUCTION

The prevalence of diabetes among expectant mothers has doubled in the past 10years. Also, the number of women entering pregnancy with pre-existing diabetes is increasing with the increasing global prevalence of diabetes. (Lawrence *et al*., 2008). This is a serious problem since diabetes in pregnancy leads to modifications in the metabolism of the mother and offspring caused by the maternal hyperglycemia. These conditions affect the fetal metabolism in-uterus and extend throughout the life of the offspring (Padilha *et al*., 2007; George *et al*., 2010). Maternal hyperglycemia give rise to complications such as macrosomia, spontaneous termination of pregnancy, still births, birth defects, premature birth, and metabolic and respiratory complications in the newborn (Maayan-Metzger, 2009). These teratogenic effects of diabetic pregnancy are due to hyperglycemia during the first crucial trimester of pregnancy when the fetus' vital organs are developing, and can affect any developing organ system. (Eriksson *et al*., 1996; Lawrence *et al*., 2008).

The effect of diabetes in pregnancy lasts for generations. An extensive study over several generations demonstrated a predominance of type II diabetes in great-grandmothers of patients with infantile onset of diabetes on the maternal side (Dörner *et al*,. 1984, 1987). In other words, diabetic pregnancy can affect the growth and metabolism of descendants and subsequent generations (Buzinaro *et al*., 2008; Simeoni and Barker, 2009). Despite considerable progress in the treatment of diabetes by modern drugs, search for newer drugs continues because the existing synthetic drugs have several limitations and are costly. A number of plants indigenous to Nigeria have been studied, and found to have hypoglycemic effects. These effects were traced to phytochemicals like alkanoids called active principles that can be extracted from plants (Ojewole, 2006; Osadebe *et al*., 2004). One of such anti-diabetic plants is *Garcinia kola*, commonly known as Bitter kola. It is an evergreen tree, indigenous to sub-Saharan Africa and belongs to a family of tropical plants called Guttifera (Ofusori *et al*., 2008). The aim of this study is to determine the effect of consumption of *Garcinia kola* extract by diabetic pregnant rats on the pregnancy outcome and early postnatal development of their offspring.

MATERIALS AND METHODS

Fresh seeds of *Garcinia kola* were obtained from local famers in Nsukka Enugu state. The fresh seeds of *Garcinia kola* were weighed, peeled, cut into bits and allowed to air dry for six weeks. The dried seeds was weighed, milled into a fine powder and stored in a dry place.1000g of this powdered *Garcinia kola* seed was dissolved in 5litres of distilled water, and allowed to stay for about 48 hours during which it was intermittently shaken vigorously. At the expiration of the 48 hours, it was filtered with muslin cloth and then with Whatman's No. 1 filter paper. The filtrate was freeze dried using YORCO Lyophilizer (York Scientific Industries Pvt Ltd.). The dried extract was reconstituted in freshly prepared normal saline (1g of extract in 10ml of normal saline) for administration to test animals. It was stored in an air tight container in the refrigerator. Forty five (45) healthy adult female rats of weight between 120 and 130g from the animal house of University of Nigeria Enugu Campus were used for the study. They were weighed, randomly assigned into metallic cages, kept in a room where a 12-h light/dark cycle was maintained, and were allowed free access to livestock feed (Top Feeds, Nigeria Ltd), the rats were allowed for 1 week to acclimatize before the commencement of the study. Forty (40) of the female rats were randomly selected and housed two per cage with a matured non-diabetic male rat of proven fertility male (2 females with 1 male in a cage) for a period of 4 days, to ensure that all the female rats get pregnant. Vaginal smear was examined under a microscope every morning, and a successful mating was ascertained by the presence of sperm cells and denotes day one of pregnancy. Diabetes was induced in twenty (20) female rats randomly selected from the forty (40) that are pregnant, by intra peritoneal injection of Alloxan hydrate (Qualikems Fine Chemicals Pvt. Ltd., India) (80 mg/kg of body weight) as a single dose after an overnight fast on the day 3 of pregnancy. The rats were fasted overnight (about 9 hours to 12 hours fasting), weighed on the induction morning and the weight used to determine the quantity of alloxan to be given. The Alloxan hydrate was dissolved in freshly prepared normal saline (1g in 20ml) in an eppendorf tube, and was given to the rats by intra-peritoneal injection, at a dosage of 80mg/kg of body weight. The remaining twenty rats were injected with 0.3ml of normal

saline. Induction of diabetes was confirmed after 48 hours by determining glucose levels in the blood taken from the tail vein of the rats using a glucometer. This study was conducted according to the Organization for Economic Cooperation and Development (OECD) revised up and down procedure for acute toxicity testing (OECD, 2001). A limit dose of 2000 mg/kg body weight of the aqueous extract of *Garcinia kola* seed was used for this study. The limit dose was performed using 5 healthy adult female rats. The rats were fasted overnight from food but not water prior to dosing and then weighed before the extract was administered orally in a single dose. The limit dose of 2000 mg/kg body weight of the aqueous extract was given to the first rat and the rat was observed for mortality and clinical signs for the first hour, then hourly for three hours and then periodically for 72hours. Other rats were subsequently dosed sequentially at 48 hours interval. The LD_{50} is predicted to be above 2000 mg/kg body weight if three or more rats survived. The twenty (20) pregnant but non diabetic rats were randomly assigned into four (4) groups of five (5) rats each. Each animal in the treatment groups was administered a volume of the extract in accordance with the dosage for its group, for a period of the pregnancy duration. The animals in the control groups were administered 0.2mls of normal saline for the same number of days. The extract administration was discontinued after delivery. Upon delivery, sixteen (16) offsprings and four (4) mothers were randomly selected per group. Four (4) offsprings were randomly assigned to each mother. The mothers reared the offsprings till the 21st day after birth (weaning day). The weight of the rats was measured with a spring balance on the 1st day of pregnancy, and then every 7th day (week) till the end of the pregnancy. The 3 weeks of pregnancy represents the 3 trimesters of pregnancy. Blood samples were collected 48hours following induction and then every 7th day (week) of pregnancy till the end of the pregnancy by piercing the tail vein of the rats. The 3 weeks of pregnancy represents the 3 trimesters of pregnancy. The blood sample collected was analyzed for blood glucose concentration using One Touch® Ultra™ glucometer (Life Scan Inc. Milano, Italy). Upon delivery, the litter size and the litter weight of the offsprings were determined. The body weight of the offspring was also studied on day 7, day 14 and day 21after birth

(weaning day). On the 21st day after birth (weaning day) also, blood samples were collected from the tail veins of the offsprings. The glucometer was used to analyze for blood glucose concentration.

RESULTS

From this study, it was observed that the animals were generally dull and slightly recumbent after administration of the extract, but became normal after about 30 minutes to 1 hour. None of the 5 rats died or showed any sign of toxicity at the limit dose of 2000mg/kg/oral in the first 48 hours and no evidence of toxicity was noted during the period of observation. The LD_{50} of aqueous extract of *Garcinia kola* seed in rats was therefore taken as above 2000mg/kg/oral.

Table 1: The result of acute toxicity (LD_{50}) test

Dosage	Survival Rate
500mg/kg	100%
1000mg/kg	100%
2000mg/kg (limit dose)	100%

Table 2 and 3 summarizes the general physical observations made on the non diabetic and diabetic pregnant rats used in the study and their offsprings during the course of the study.

All the rats in all the groups delivered except for 1 rat (20%) in the diabetic pregnant control group that did not deliver and was discarded. Among the 21 offsprings of the diabetic pregnant control group, there were two (2) still births (9.5%), and three (3) (14.3%) died during the neonatal period. Among the 30 offsprings of diabetic pregnant test group 1 (100mg/kg), there was one (1) still birth (3.3%), and one (1) (3.3%) died during the perinatal period. No still birth or perinatal death was recorded in the other groups, and no physical anomaly (physical defect) was observed among the litters of the entire experimental groups.

Table 2: Physical observations on the non-diabetic rats used in the study and their offsprings

Non diabetic Rats					
Groups	**Number of rats that delivered [n (%)]**	**Litter**	**Total stillbirths [n(%)]**	**Perinatal death in Offsprings [n (%)]**	**Physical anomally in offsprings**
Control	5 (100%)	40	NIL	NIL	NIL
100mgkg-1	5 (100%)	40	NIL	NIL	NIL
200mgkg-1	5 (100%)	40	NIL	NIL	NIL
300mgkg-1	5 (100%)	40	NIL	NIL	NIL

Table 3: Physical observations on the non-diabetic rats used in the study and their offsprings

Diabetic Rats					
Groups	**Number of rats that delivered [n (%)]**	**Litter**	**Total stillbirths [n(%)]**	**Perinatal death in Offsprings [n (%)]**	**Physical anomally in offsprings**
Control	4 (80%)	21	2 (9.5%)	3 (14.3%)	NIL
100mgkg-1	5 (100%)	30	1(3.3%)	1 (3.3%)	NIL
200mgkg-1	5 (100%)	35	NIL	NIL	NIL
300mgkg-1	5 (100%)	40	NIL	NIL	NIL

DISCUSSION

The method used for the induction of diabetes was effective in producing hyperglycemia in all (100%) of the alloxan-treated rats. This is in support of the existing procedure that alloxan induces diabetes in rodents. The acute toxicity value of greater than 2000mg/kg was obtained after the administration of the aqueous extract of *Garcinia kola* seed in rats. This is an indication of the extracts' none or low toxicity because an LD50 of >2000mg/kg is classified as practically non-toxic (U.S.EPA, 2006). In this study, *Garcinia kola* extract significantly reduced the pregnancy induced weight gain among the non-diabetic pregnant rats in a dose dependent manner (Table 2).This

supports the observation made by Deshmukh *et al*., (2008) who observed a that *Garcinia kola* extract reduces weight gain in pregnancy. A statistically significant reduction in weight gain in pregnancy was observed among the rats in the diabetic pregnant control group when compared to the control in this study (Table 3). This is in agreement with Lin *et al*., (1995), who observed a statistically significant reduction in weight gain in pregnancy among diabetic pregnant rats. *Garcinia kola* was able to reverse this diabetes induced significant reduction in pregnancy weight gain in a dose dependent manner in this study (Table 2).This may be due to the insulinogenic effect of kolaviron an active principle in *Garcinia kola* which is believed to possess pancreatic beta cells re-generating, insulin releasing effects and fighting the problem of insulin resistance (Wielinga *et al*., 1982; Hongxiang *et al*., 2009). This makes the available glucose utilizable by the tissues for energy generation and anabolic processes leading to growth. The result of this study indicates that diabetes in pregnancy significantly reduced the litter size in rats (Table 3).A possible explanation to this may be that maternal hyperglycemia during pregnancy may give rise to miscarriage (Melamed and Hod, 2009). This may be caused by the altered antioxidant status leading to oxidative stress in the fetus of animals and humans (Grissa *et al*., 2007). Since rats are multiparous, this tendency may have caused the loss of some of the fetuses, leading to the observed reduced litter size. *Garcinia kola* extract significantly increased the litter size among the diabetic pregnant rats in a dose-dependent manner in this study (Table 2 & 3).The likely explanation for the observed potency of the extract in reversing this reduced litter size may be the effects of kolaviron and ascorbic acid (constituents of *Garcinia kola*) that have anti-oxidative stress action as observed in previous studies (Farombi *et al*., 2004; Adegoke *et al*., 1998). Another possible explanation could be due to the glucose lowering effect of this extract since it leaves little or no excess glucose for the offsprings to absorb, thus reducing the tendency to induce oxidative stress. The observations of this study suggest that *Garcinia kola* may serve as a potent hypoglycemic agent for the management of diabetes in pregnancy. This study also suggests that *Garcinia kola* can reverse the adverse effect of diabetes in pregnancy on both the

mother and the offspring in childhood.

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