***EFFECTS* OF AQUEOUS EXTRACT OF BITTER KOLA(*GARCINIA KOLA*) ON THE PREGNANCY OUTCOME AND EARLY POSTNATAL DEVELOPMENT OF THE OFFSPRINGS OF DIABETIC PREGNANT RATS**

**SUBMITTED**

**BY**

**EZE, VIVIAN .N.**

**GOU/12/1355**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF**

**BACHELOR OF SCIENCE (B.SC) IN BIOTECHNOLOGY DEPARTMENT OF**

**BIOLOGICAL SCIENCE, GODFREY OKOYE UNVRSITY, ENUGU STATE.**

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**JULY,2016**

**APPROVAL PAGE**

This project has been read and approved for the department of Biological science, Facult of Natuaral and Applied sciences, Godfrey Okoye University, Enugu in partial fulfilment of requirement for the award of Bachelor of Science(B.Sc) degree in biotechnology by;

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Project Supervisior Date

CERTIFICATION

This is to certify that the report entitled,” the the antimicrobial effect of *G.* kola on the treatment

of streptococcus pyogene” submitted by Eze, Vivian N; Gou/12/1355 in partial fulfilment of the

requirements for the award of bachelor of science in biotechnology at Godfrey Okoye

University, Enugu was prepared by her, under my supervision and guidance.

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Head of Department

DEDICATION

I dedicate this project to almight for his mercy and upliftment.

ACKNOWLEDGEMENT

First and foremost I thank God Almighty who strengthens me to accomplish my project.

I own a great deal to my project supervisor DR.(Mr) for her guidance, advice contribution at every stage of this project. My profound gratitude goes to our HOD Dr (Mrs) head of Biological Science Department. And also to appreciate our Vice Chancellor prof. Aneke, Christian and to the Dean of studies faulty of Natural And Applied Sciences Mr. to all the lecturers in the Department of Biological Sciences am grateful for your contributions and success to my academic life.

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TOPIC : EFFECT OF AQUEOUS EXTRACT OF BITTER KOLA(*GARCINIA KOLA*) ON THE PREGNANCY OUTCOME AND EARLY POSTNATAL DEVELOPMENT OF OFFSPRINGS OF DIABETIC PREGNANT RATS.

**ABSTRACT**

Increased oxidative stress arising from the maternal hyperglycemic-induced disturbances in fetal metabolism has been suggested to play a role in the pathogenesis of disturbed embryogenesis in diabetic pregnancies. Maternal diabetes in pregnancy and the associated hyperglycemia is also believed to expose the fetus to disturbed metabolic conditions in-utero creating a ‘metabolic memory’ that programs the fetus for glucose intolerance, diabetes mellitus and obesity later in life. Bitter *kola* (*Garcinia kola*) is a medicinal plant with a wide range of pharmacological effects including ant diabetic 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 alloxan diabetiPc and non-diabetic Wister rats was studied. Forty (40) nulliparous female Wister 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 ofBitter 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 kolamay have a protective effect against the adverse effects of diabetes in pregnancy on both the mother and the offspring.

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**LIST OF ABBREVATIONS**

2-A AS - 2 Amino-adiposemialdehyde

2-AAF - 2-Acetyl Amino Fluerene

ANOVA - Analysis of Variance

BDCP - Bioresources Development and Conservation Programme

BHA - Butylated Hydroxyanisole

CCl4 - Carbon Tetrachloride

DNA - Deoxyribo Nucleic Acid

EDTA - Ethylendiaminetetraacetic Acid

eg - Example

ENDO 111 - Pyrimidine Endonuclease III

FEV1 - Forced Expiratory Volume in one second.

FPG - Formamidopyrimidine Glycosylase

FVC - Forced Vital Capacity

g - Grams

GDM - Gestational Diabetes Mellitus

GGS - Gamma Glutamyl Semialdehyde

GSH - Glutathione

H2O2 - Hydrogen Peroxide

HCA - Hydroxycitric Acid

HCL - Hydrochloric Acid

HepG2 - Human Hepatoma Cell Line 2

HMG –COA - 3-hydroxy -3-Methlglutaryl Coenzyme A

IOP - Intra Ocular Pressure

Kg - Kilograms

LDL - Low Density Lipoproteins.

LD50 - Lethal Dose 50 15

LGA - Large for Gestational Age

MBC - Minimum Bactericidal Concentration

Mg/dl - - Miligram Perdecialiter

MIC - Minimum Inhibitory Concentration

ml - Millitres

MRSA - Methicillin Resistant Staphylococcus Aureus

NAPQI – N -acetyl – P-benzoguinoneimine

NPH - Neutral Protamine Hagedorn

OECD - Organisation for Economic Cooperation and Development

PEFR - Peak Expiratory Flow Rate.

RBC - Red Blood Cells.

SEM - Standard Error of Mean

SGOT - Serum Glutamic Oxaloacetic Transaminase.

SGPT - Serum Glutamic Pyruvate Transaminase

VRE - Vacomycin Resistant Escherichia Coli.

**CHAPTER ONE**

**INTRODUCTION**

**1.1 INTRODUCTION**

Compelling evidence suggests that exposure to an adverse fetal environment may enhance susceptibility to a number of chronic diseases in the future life of the offspring(Buzinaro et al, 2008; Simeoni and Barker, 2009).

Diabetes mellitus is a condition that occur during pregnancy that can substantially influence the development of the offspring in utero and postnatally. Diabetes mellitus is now a pandemic, affecting about 10million Nigerians (Ogbera *et al*, 2005) and about 350 million people worldwide (Ezzati *et al*, 2011) among who are pregnant women. It is well documented that the combined stress of diabetes mellitus and pregnancy creates a metabolic environment that is often life threatening to both the mother and the fetus (Freinkel, 1980; Metzger, 1991).

Hence, pregnancy among women that have pre-existing diabetes or gestational diabetes is associated with increased rate of adverse outcome for both mother and fetus (Kingsley, 2007; Shefali *et al*., 2006).

This is primarily due to altered maternal intrauterine environment, creating a situation in which the fetus is exposed to abnormal metabolic substrate (glucose) levels (Van Assche *et al*, 1991). There is an increased placental transfer of glucose from mother to fetus because of increased availability at the maternal site (Thomas *et al*, 1990). The compromised metabolic state of the fetus subsequently precipitates a variety of complications associated with ‘‘fuel-mediated teratogenesis’’ (e.g., hyperglycemia, hyperinsulinemia and macrosomia) (Freinkel, 1980; Metzger, 1991). One particularly devastating effect of diabetic pregnancy is that these conditions affect the fetus not only in utero, but also extend throughout the life of the offspring (Padilha *et al*, 2007; George *et al*, 2010). Additionally, maternal hyperglycemia stimulates abnormal fetal growth (Aberg *et al*, 2001) due to the greater availability of glucose in the blood flow (Maayan-Metzger *et al*, 2009), and this high weight fetus carries a high risk for 17

developing insulin resistance, glucose intolerance, obesity, and type 2 diabetes mellitus in childhood, adolescence and adulthood (Buzinaro *et al*, 2008; Simeoni and Barker, 2009)

The chances of reducing this poor outcome of pregnancy among diabetics are intricately related with the level of glycemic control (Shefali *et al*., 2006).

The fact that the economic cost of managing diabetes mellitus is high confers a very important role to medicinal plants in the management of diabetes mellitus especially in developing countries where resources are meager.

Consequently, 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(Ojewale, 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 seed is a masticatory, used for traditional hospitality in cultural and social ceremonies. Every part of the plant has shown to be of medicinal importance and has a wide range of medicinal effects, hence the name ‘wonder plant’ as it is commonly called.

Among the litany of its medicinal effects are; antidiabetic effects (Iwu *et al*, 1990), weight reducing effets (Koshy *et al*., 2001), leptin like action (Hayamizu *et al*., 2003), antihepatotoxic effects (Akintonwa and Essien, 1990), antioxidative stress and anti DNA 18

damage (Farombi, *et al*., 2004), detoxification of the toxic effects of other chemicals (Esimone *et al.*, 2002; Nwokocha *et al*, 2011), etc.

**1.2 JUSTIFICATION FOR THE STUDY**

Diabetes mellitus is fast becoming the most common type of disease in school children (Pontiroli, 2004). This may be as a result of the reported more than doubling in therate of diabetes among expectant mothers between 2002 and 2008 (Lawrence *et al*., 2008).

The economic cost of managing diabetes is high. As a result, in the developing countries where resources are meager, there is a shift from contemporary to orthodox medicine since medicinal plants are relatively easier to find and less expensive alternative.

Coincidentally, some of the commonly consumed plant materials in Nigeria have been found to poses anti-diabetic properties. One of such anti-diabetic plantmaterials Bitter kola seed is used as a stimulant. It is also taken by pregnant women to stop nausea.

Although Bitter kolaseed is a known anti-diabetic, there is paucity of data on;

(1) Its effects on pregnancies complicated by diabetes mellitus

(2) Early postnatal development of the offspring’s of such pregnancies.

Hence, the present study was therefore designed to investigate these.

**1.3 AIM**

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.

**1.4 OBJECTIVES**

To determine the effect of consumption of Bitter kolaextract by diabetic pregnant rats on;

i. The litter size.

ii. The early postnatal growth of the offsprings (from birth to weaning).

iii. The glucose profile of the offsprings at weanin

**1.5 OPERATIONAL DEFINITION OF TERMS**

**Alloxan diabetes:** A type of diabetes induced in rodens by the injection of alloxan hydrate. 20 **Pregnancy weight gain:** The weight gain during pregnancy.

**Programming:** The process whereby a stimulus or stress at a critical period of development of the rats results in a lasting or lifelong effect.

**Teratogenic:** Capable of causing developmental abnormalities in the fetus  **Diabetic pregnancy:** Pregnancy complicated by diabetes mellitus irrespective of the type.

**Early Postnatal development:** This is the developmental processes that occur in the offsprings from the time of birth to the weaning day.

**Gestation period:** The period (in days) between the time when spermatozoa were first seen in vaginal smear and the time of delivery.

**Glucose drain:** The transfer of glucose from the maternal to fetal blood.

**In-utero:** Events occurring inside the uterus.

**Litter Size:** The number of offspring delivered by a pregnant rat.

**Litter weight:** The weight of the offsprings at birth (g).

**Perinatal:** Events that occur around the time of birth.

**Pregestational (Pre-existing) diabetes:** Diabetes existing before pregnancy, irrespective of the type.

**Pregnancy weight gain:** The weight gain during pregnancy.

**Programming:** The process whereby a stimulus or stress at a critical period of development of the rats results in a lasting or lifelong effect.

**Teratogenic:** Capable of causing developmental abnormalities in the fetus..

**CHAPTER TWO**

**LITERATURE REVIEW**

**2.1 DIABETIC PREGNANCY**

Diabetic pregnancy refers to both pre-gestational and gestational diabetes. Gestational diabetes (GDM) is a type of diabetes diagnosed after the onset of pregnancy, while pre-gestational diabetes occurs when women with pre-existing type 1 or type 2 diabetes conceive.

The prevalence of diabetes among expectant mothers has more than 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-utero 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).

**2.2 ALLOXAN DIABETES IN PREGNANCY**

Studies in humans that explore the responsible mechanisms for alterations caused by diabetes in pregnancy are limited not only by ethical reasons but also by the multiplicity of uncontrolled variables that may modify the intrauterine environment (Lopez-Soldado and Herrera, 2003). Thus, there is a need for appropriate animal models for a better understanding of the diagnosis, pathophysiology and treatment of diabetes. Diabetes can be produced in experimental animals surgically by pancreatectomy (Foglia *et al*, 1967) or chemically by administration of streptozotocin (Brenna *et al*, 2003) or alloxan (Goldner and Gomori, 1944). Additionally, there are models that have been developed to reproduce the clinical conditions of type 1, type 2 and gestational diabetes in laboratory animals, depending on method and timing of induction.

Alloxan induction is easy to perform, results in a high percentage of diabetic rats, is similar to human disease and has been widely used in our institution.Alloxan is an oxygenated pyrimidine derivative present as alloxan hydrate in aqueous solution. It is a toxic analogue of glucose which selectively destroys the insulin producing pancreatic beta cells especially in rodents causing an insulin dependent type of diabetes mellitus called alloxan diabetes. This type of diabetes has characteristics similar to type 1 diabetes in humans (Lenzen, 2008).

In order to reproduce the clinical conditions ofdiabetes in pregnancy, experimental models are used to produce severe (glycemia>300 mg/dL), and mild diabetes (glycemia between 120 and 300 mg/dL) depending on dosage of alloxan administered. Alloxan diabetes in pregnancy produces similar effects as diabetic pregnancy (Rees and Alcolado, 2005).

**2.3 PATHOPHYSIOLOGY OF DIABETES IN PREGNANCY**

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells. Therefore deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus.

During a normal pregnancy, each meal causes a rise in blood glucose level. This in turn sets in motion a complex series of hormonal actions, including the secretion of pancreatic insulin, placental hormones, glucagon, somatomedins, and adrenal catecholamines. These adjustments ensure that an ample, but not excessive, glucose-'drain' to the fetus, and supply of glucose to the mother. Compared with non-pregnant subjects, pregnant women tend to develop hypoglycemia (plasma glucose mean = 65-75 mg/dL) between meals and during sleep. This occurs because the fetus continues to draw glucose across the placenta from the maternal bloodstream, even during periods of fasting. Interprandial hypoglycemia becomes increasingly marked as pregnancy progresses and the glucose demand of the fetus increases. Maternal body insulin requirement increases as pregnancy advances. This is because the levels of placental steroid and peptide hormones (eg, estrogens, progesterone, and chorionic somatomammotropin) rise linearly throughout the second and third trimesters. These hormones confer increasing tissue insulin resistance as their levels rise, the demand for increased insulin secretion with feeding escalates progressively during pregnancy, and by the third trimester, 24-hour mean insulin levels are 50% higher than in the non-pregnant state.

When the maternal pancreatic insulin response is inadequate, there are recurrent episodes of maternal postprandial of hyperglycemia. This in turns causes fetal hyperglycemia as a result of a marked increase in the placental transfer of glucose from mother to fetus because of increased availability at the maternal site. These postprandial episodes of hyperglycemia are the most significant source of the accelerated growth exhibited by the fetus. This is because these episodes of hyperglycemia are accompanied by episodic fetal hyperinsulinemia which promotes excess nutrient storage, resulting in macrosomia. The mechanisms by which maternal diabetes during pregnancy may lead to congenital abnormalities (teratogenicity) in the fetus are not fully understood. One of the earliest suggestions is the involvement of oxidative stress (Baynes and Thorpe, 1999). Fetuses from mothers with gestational diabetes are at increased risk of developing platelet hyperag gregability and oxidative stress (Kamath *et al*, 1998).

The energy expenditure associated with the conversion of excess glucose into fat causes depletion in fetal oxygen levels leading to hypoxia. The induced oxidative stress leads to alteration in antioxidant enzymes activities (Dincer *et al*, 2002), impaired glutathione metabolism (McLennan *et al*, 1991) and decreased ascorbic acid levels (Young *et al*, 1992). This in turn, induces the production of highly reactive oxygen radicals, being toxic to cells, particularly to the plasma membranes where these radicals interact with the lipid bilayer. In other words, maternal diabetes in animals as well as in humans is associated with altered antioxidant status (Yessoufou *et al*, 2006; Grissa *et al*, 2007) in both the mother and offspring. The mechanisms by which maternal diabetes during pregnancy predisposes the offspring to disease in childhood and later in life are not fully understood. Some people suggest that the “metabolic memory” concept is the mechanism by which maternal diabetes during pregnancy may lead to disease in the offspring at childhood and adulthood**.** The hypothesis on fetal origin of diseases in later life suggests that the condition to which a fetus is exposed to at the critical and delicate period of fetal development has long-term impacts on fetus. This was previously described and established as “fetal programming” by Hales and Barker (2001), but now termed as new concept of “metabolic memory”. In the same line, the metabolic abnormalities in diabetic pregnant women create an in-utero environment around the fetus which programs it for diseases later in life (Hales and Barker, 2001; Dorner *et al*, 1994). This in-utero programming seems to create a kind of “metabolic memory”, since physiological anomalies of gestational period are responsible for the onset of diseases in offspring later, such as type 2 diabetes and macrosomia or obesity associated with metabolic syndrome. Several alterations in carbohydrate and lipid metabolism, observed in infants of diabetic mothers at birth also persist postnatally.

Supporting the metabolic memory concept, a study by Franke *et al*. (2005)showed that diabetic pregnancy in rats alter the differentiation of hypothalamic neurons of newborns. The exposure to the intrauterine environment of diabetic pregnancy milieu causes a defective programming of hypothalamic neurons with increased number of neuropeptide-Y hormones. These alterations may increase the risk of trend in high food-taking, overweight, obesity and diabetogenic status in offspring. The alterations of hypothalamic neurons may be avoided by normalizing the glycemia among diabetic pregnant rats (Franke *et al*, 2005).

**2.4 FETAL COMPLICATIONS OF DIABETES PREGNANCY**

Diabetes mellitus in pregnancy can present some particular challenges for the mother and child causing a litany of complications for both the mother and the offspring. These complications are caused by increased production of reactive oxygen species, following an increased glucose metabolism in embryo cells. This increases oxidative stress through a complex network of altered biochemical pathways, which combine to increase reactive oxygen species production and to decrease availability of GSH (glutathione) for free radical scavenging. (Eriksson and Borg, 1993)

**2.4.1 Miscarriages**

In all women with preexisting diabetes mellitus, there is a 9-14% rate of miscarriage. Current data suggest a strong association between the degree of glycemic control before pregnancy and the miscarriage rate. Suboptimal glycemic control has been shown to double the miscarriage rate in women with diabetes (Melamed and Hod, 2009). A correlation also exists between more advanced diabetes and miscarriage rates. Patients with long-standing (>10 years) and poorly controlled diabetes (glycohemoglobin exceeding 11%) have been shown to have a miscarriage rate of up to 44%. Conversely, excellent glycemic control normalizes the miscarriage rate.

**2.4.2 Birth defects and Still births**

It is well established that the fetus of the mother with poorly controlled diabetes mellitus is at increased risk for neonatal morbidity and mortality (Diamond, *et al.*, 1987). Diabetic pregnancy increases the risk ofmiscarriage, stillbirth, congenital malformations, placentalabnormalities, and intrauterine malprogramming (Melamed and Hod, 2009).

Major birth defects occur in 1-2% of the non diabetic population. Women with overt diabetes and suboptimal glycemic control before conception, has a 4- to 8-fold increased likelihood of a structural anomaly. Initial reports showed anomaly rates as high as 18% in women with preexisting diabetes mellitus (Becerra *et al*, 1990), but more recent studies, showed anomaly rates of 5.1 - 9.8% in patients who received more aggressive preconception and first trimester management ( De Valk *et al*, 2006; Dunne *et al*, 2003) Clinical trials of intensive metabolic care have demonstrated that malformation rates similar to those in the non-diabetic population can be achieved with meticulous preconceptional glycemic control. Subsequent trials comparing a preconceptional intensive metabolic program to standard treatment have demonstrated lowered rates of perinatal mortality (0% vs 7%) and congenital anomalies (2% vs 14%). (Dunne *et al*, 2003).

**2.4.3 Growth restriction**

Although most fetuses of diabetic mothers exhibit growth acceleration, growth restriction occurs with significant frequency in pregnancies in women with preexisting type 1 diabetes. The most important predictor of fetal growth restriction is underlying maternal vascular disease. Specifically, pregnant patients with diabetes-associated retinal or renal vasculopathies and/or chronic hypertension are most at risk for growth restriction (Smelter and Bare, 2000)

**2.4.4 Obesity**

Excessive body fat stores, stimulated by excessive glucose delivery during diabetic pregnancy, often extends into childhood and adult life. Approximately 30% of fetuses of women with diabetes mellitus in pregnancy are large for gestational age (LGA). In preexisting diabetes mellitus, this incidence appears to be slightly higher (38%) (Ehrenberg *et al*, 2004).

**2.4.5 Macrosomia**

Macrosomia is the main and the most commonly reported adverse outcome of diabetes in pregnancy (Mitanchez, 2010). Maternal diabetes is characterized by an increased placental transport of glucose and othernutrients from the mother to the fetus, resulting in macrosomia (Van Assche *et al*, 2001). Macrosomia is usually defined in humans as birthweight above either 4kgor birth weight above the 95th percentile of the gestational age. Macrosomia occurs in 15-45% of babies born to diabetic women, a 3-fold increase from normoglycemic controls (Ategbo *et al*, 2006; Grissa *et al*, 2007).

The macrosomic fetus in diabetic pregnancy develops a unique pattern of overgrowth; involving central deposition of subcutaneous fat in the abdominal and interscapular areas (McFarland *et al*, 1998).Skeletal growth is largely unaffected.Convincing studies haveshown that either preexisting diabetes (type 1 and type 2 diabetes) or GDM (diabetes only duringpregnancy) are associated with macrosomia (Evers *et al*, 2004; Ategbo *et al*, 2006; Grissa *et al*, 2007). Indeed, epidemiological and clinical studieshave shown that maternal type 1 diabetes during pregnancy is an important risk factor for fetalovernutrition and macrosomia, and for the development of obesity and diabetes in offspring (Evers *et al*, 2004; Giordano, 1990).

**2.4.6 Metabolic syndrome**

Population-based studies have also demonstrated long-term consequences for the offspring of gestational diabetic mothers. These progeny have an increased risk for obesity, glucose intolerance, and type 2 diabetes in later childhood and as adults(Silverman *et al*, 1995).The adverse downstream effects of abnormal maternal metabolism on the offspring have been documented well into puberty. Glucose intolerance and higher serum insulin levels are more frequent in children of diabetic mothers than in normal controls. By age 10-16 years, offspring of diabetic pregnancy have a 19.3% rate of impaired glucose intolerance (McKinney *et al*, 1999).

The childhood metabolic syndrome includes childhood obesity, hypertension, dyslipidemia, and glucose intolerance. A growing body of literature supports a relationship between intrauterine exposure to maternal diabetes and risk of a metabolic syndrome later in life (Plagemann, 2005; Eriksson *et al*, 2003). Fetuses of diabetic women that are born large for gestational age appear to be at the greatest risk (Eriksson *et al*, 2003).

**2.5 MANAGEMENT OF DIABETES IN PREGNANCY**

Proper management of diabetes in pregnancy can minimize the risks posed by glucose intolerance during pregnancy.It involves different strategies which include life style changes (dietary changes and physical activity) and pharmacological interventions (insulin therapy and oral hypoglycemic agents).

Lifestyle intervention should promote dietary carbohydrate moderation, exercise in patients and weight loss in those who are overweight.

Though there is an expanding battery of pharmacologic agents that can be used to reach glycemic targets, insulin remains the standard medication for treatment of diabetes during pregnancy. Insulins lispro, aspart, regular, and neutral protamine hagedorn (NPH) are well-studied in pregnancy and regarded as safe and effective (Cheung, 2009). The efficacy and safety of insulin have made it the standard for treatment of diabetes during pregnancy. Nevertheless, the oral hypoglycemic agents, glyburide and metformin are gaining popularity. Trials have shown these agents to be effective and no evidence of harm to the fetus has been found, although the potential for long-term adverse effects remains a concern (Cheng *et al,* 2008).

**2.6 TRADITIONAL PHYTOTHERAPY**

This refers to the medical treatment that is based exclusively on plant extracts and products. From Neolithic time some plants have been discovered to have medicinal value, (Olaleye *et al*, 2000) and provided a wide range of important drugs. Current research into drugs from plant continues to be fruitful, and have been used as a source of various drugs from which man can get a cure for almost all ailments.

70% of people living in Nigeria depend on various forms of herbal decoctions for the treatment of diseases. This situation makes it pertinent to investigate these plant extracts, which are increasingly advertised by herbalists as being effective against a myriad of diseases and disorders. A good number of them were in use long before their scientific backgrounds were discovered.

This effectiveness of these medicinal plant products has been traced to phytochemical Substances called essential/active principles which have been implicated by extraction from these natural herbs. These active principles are mostly phytochemicals like alkaloids (Ukairo, 2001).

**2.7 TRADITIONAL PHYTOTHERAPY IN THE TREATMENT DIABETES**

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. Consequently, medicinal plants play an important role in the management of diabetes mellitus, especially in developing countries where resources are meager. (Bnouham *et al*., 2006)

The herbal drugs with anti-diabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine (Wadkar *et al*, 2008). The plants provide a potential source of hypoglycemic drugs because many plants and plant derived compounds have been used in the treatment of diabetes (Osadebe *et al*., 2004). Many plants indigenous to Nigeria and from other countries have been found to have hypoglycemic (anti-diabetic) effects and reports occur in numerous scientific journals. These effects were traced to phytochemicals like alkanoids called active principles that can be extracted from plants (Ukairo, 2001). Ayurveda and other traditional medicinal system for the treatment of diabetes describe a number of plants used as herbal drugs. Hence, they play an important role as alternative medicine due to less side effects and low cost. The active principles present in medicinal plants have been reported to possess pancreatic beta cells re-generating, insulin releasing and fighting the problem of insulin resistance (Welihinda *et al*, 1982). Hyperglycemia is involved in the etiology of development of diabetic complications. Hypoglycemic herbs increase insulin secretion, enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from intestine and glucose production from liver (Hongxiang *et al*, 2009).

Plants that have antidiabetic properties include Myrcia sphaerocarpa (Matsuda *et al*., 2002), Garlic (Jelodar *et al*, 2005), Cinnamomum aromaticum (Richard, 2005), Grifola frondosa (Manohar *et al*., 2002), e.t.c

**2.8 BITTER KOLA*(GARCINIA KOLA)***

*Garcinia kola* is forest tree indigenous to sub-Saharan Africa and has been referred to as a ‘wonder plant’ because almost every part of it has been found to be of medicinal importance (Hutchinson and Dalziel, 1956). It occurs naturally from Sierra Lone to Southern Nigeria and on into Zaire and Angola, but is further distributed by man and is often found cultivated around villages. *Garcinia kola* belongs to a family of tropical plants known as Guttifera, (Plowden, 1972). It is an evergreen tree grown in the tropical rainforest of West Africa, (Burkhill, 1985; Ofusori *et al*., 2008). It grows to a height of about 30metres high, and the fruit, which is in the size of an orange, is smooth and reddish yellow with peach-like skin and yellow pulp and contains three or four seeds covered with brown seed coat (Aniche and Uwakwe, 1990). The seed is an edible nut (Ofusori *et al*., 2008), generally known and called Bitter Kola in Nigeria, and commonly called “Namiji goro” in Hausa, Orogbo in Yoruba and “Aku-ilu” in Igbo. The seed (commonly known as bitter kola, male kola or false kola) is a masticatory used in traditional hospitality, cultural and social ceremonies. Extractive of the plant have been traditionally used for ailments such as laryngitis, liver diseases and cough (Ayensu, 1978). The seeds are used to prevent or relieve colic, cure head or chest colds and relieve cough (Iwu, 1993). The seed also has anti-inflammatory, antimicrobial, antidiabetic and antiviral as well as antiulcer properties (Ibironke *et al*, 1997).

**2.8.1 Constituents of *Garcinia kola***

Phytochemical and biochemical studies of *Garciniakola*showed the presence of sterols, terpenoids, flavonoids, glycosides, pseudotannins, saponin, proteins and starch (Igboko, 1983; Braide and Vitrotio, 1989).Maduniyi (1983) reported that some workers isolated kolanone, a poly-isoprenyl-benzophenone compound from the fruit pulp.

*Garcinia kola* is a reasonable source of ascorbic acid, some micro-elements including nitrogen, potassium, phosphorus, magnesium and calcium, a trace amount of chromium (Eka, 1984).Another medicinal constituent of *Garciniakola* is hydroxycitric acid (HCA) (Jena *et al*., 2002). Xanthones, xanthone derivatives, and polyisoprenylated benzophenones have also been isolated from Garcinia *kola* (Masullo *et al*., 2008; Koshy *et al*., 2001).

*Garcinia kola* also contains toxic substances such as tannins, phytic and hydrocyanic acids at a low concentration. Other constituents include Ash and Crude protein, Crude Fiber, Crude Lipid, water – soluble oxalate, terpenoids and fat (Aniche and Uwakwe, 1990).

**2.8.2 *Garcinia kola* and Pregnancy and Lactation**

Information regarding safety and efficacy in pregnancy and lactation is lacking. One animal study in rats documented decreased maternal body weight gain during gestation (Deshmukh *et al*., 2008). In another study, Akpantah*et al* (2005) studied the effect of *Garcinia kola* extract consumption by pregnant rats on the number oftotal implantation, resorption, weight of fetus, gross malformations, fetal parameters (fetal number, weight, crown-rump-length,and length of umbilical cord, and placental weight) on day 21 of gestation. They observed that all dams survived to theirscheduled termination day. There were noabortions, no early deliveries and no death ofanimal during the study. They also observed a significant reduction in fetal weight, but parameters for growth(crown-rump, placenta weight) were not affected, and there were no resorption and nopost-implantation sites.

**2.8.3Some Previous Studies and Case Reports on *Garcinia kola***

Many pharmacological effects of *Garcinia kola* have been extensively studied and well documented in the medical journal. Among them are;

* **Antidiabetic (Blood Glucose Lowering) Effect**

There is a vast documentation of the antidiabetic effects of *Garcinia kola*. Bioflavonoids obtained from *Garcinia kola* has been shown to have significant hypoglycemic effect when administered intraperitoneally at 100mg/kg body weight to normal and alloxan diabetic rabbits (Iwu *et al*., 1990). Another study, by Adaramoye and Adeyemi, (2006) demonstrated the hypoglycemic and hypolipidemic effects of *Garcinia kola*in streptozotocin induced diabetes mellitus in rats.

Hydroxycitric acid (HCA)from *Garciniakola* delayed and reduced intestinal glucose absorption in rats; the treatment causes delayed intestinal absorption of glucose (Wielinga *et al*., 2005).

* **Weight Loss and Lipid-Lowering Effect**

The medical literature is rich in research documentation on the weight loss and lipid-lowering activity of the plant.

In two invitro experiments using the human hepatoma cell line HepG2, overnight exposure to *Garcinia kola* extract caused an upregulation of low-density lipoprotein (LDL) receptor activity and an upregulation of the level of HMG-CoA reductase, resulting in decreased cholesterol synthesis (Berkhout *et al*., 1990).

Flavonoids from the plant reduced lipid levels in normal and hypercholesterolemic rats. Reductions were also documented in triglycerides, phospholipids, and free fatty acids (Koshy *et al*., 2001).

*Garcinia kola* extract was able to stop dexamethasone induced elevation in lipid profiles amongrats (Mahendran and Devi, 2001).

The suggested mechanism of action for HCA and flavonoids weight and lipid profile reduction action may involve inhibiting lipogenesis, increasing lipid oxidation, and reducing food intake (Ohia *et al*, 2002; Mattes and Bormann, 2000). The food intake reduction may be due to the leptin like action of HCA as reported by Hayamizu *et* *al*(2003).

Another study that recorded weight reduction as a result of extracts from the plant is Girola *et al*., (1996).

* **Anti-Oxidative Stress (Antioxidant) Effects**

It was discovered that kolaviron from *Garciniakola* at 200mg/kg body weight significantly reduced a tetra-butyl hydro peroxide induced in 2-amino-adiposemialdehyde (2-AAS) a maker of protein oxidation in both plasma and liver, hence decreasing oxidative damage to DNA in the liver. (Farombi *et al*., 2004)

In another study carried out by Farombi and another group of researchers, kolaviron from *Garciniakola* (100mg/kg body weight for 1week) protected rat liver cells against H2O2 induced DNA strand breaking, oxidized purine (Formamidopyrimidine glycosylase (FPG) and pyrimidine (endonuclease III (ENDO III) sites bases, sensitive sites both in rat liver and human lymphocytes, and Fe3+/EDTA/ ascorbate-induced malondialdehyde formation and protein oxidation. Gamma glutamyl semialdehyde (GGS) and 2- amino adiposemialdehyde (2-AAS) were used as biomarkers of oxidative damage to protein.

They suggested that kolaviron exhibits protective effects against oxidative damage to molecular targets through the scavenging of free radicals or iron binding.

Kolaviron may therefore be relevant in the chemoprevention of oxidants induced genotoxicity and possibly human carcinogenesis. (Farombi *et al*., 2004) The *Garcinia* fruit also contains xanthones, which inhibit pre-neoplastic lesions in mammary and colon cancer. The xanthones may also induce apoptosis in mouth, leukemia, breast, gastric, and lung cancer cell lines in vitro (Mazzio and Soliman, 2009). Another study that found that supplementation with *Garciniakola* can reduce oxidative damage is Yonei *et al*, (2008).

* **Detoxification of the Toxic Effects Of Toxicants.**

Nwokocha *et al,* (2011) observed a detoxification of lead poisoning by G*arcinia kola* in wistar rats. It displayed antihepatotoxic and antihaematotoxic effects in lead poisoned wister rats.

*Garciniakola* extract has also been shown to cause a reduction in the level of liver enzymes SGOT and SGPT in Paracetamol induced hepatotoxicity in rats. This is believed to be due to the inhibition of cytochrome P-450 which normally converts paracetamol to the toxic intermediate metabolite N-acetyl-p-benzoquinoneimine (NAPQI) (Akintonwa and Essien, 1990). In another study, kolaviron a *Garciniakola* extract showed antihepatotoxicity in CCl4 induced hepatotoxicity in rats (Braide, 1991), while Adegoke *et al*., 1998 showed that *Garciniakola* extracts inhibited invitro lipid peroxidation in rat liver homogenate in a dose dependent manner.

*Garciniakola* extract also protected rat liver from 2-acetyl amino fluerene (2-AAF) induced hepatotoxicity and lipid peroxidation, and is as effective as BHA. It was described as an invivo natural antioxidant and an effective hepatoprotective agent. (Farombi *et al*., 2000).

Another study also portrays kolaviron an extract of *Garciniakola* as an effective chemo preventive agent against aflatoxin B-1 induced genotoxicity and hepatic oxidation in rats and thus may qualify for clinical trials in combating aflatoxicosis in endemic areas of aflatoxin contamination of foods. (Farombi *et al*., 2005). 39

The role of *Garciniakola* extracts as a poison antidote has been demonstrated as early as 1985, when kolaviron reduced lethal poisoning of mice by phalloidin (Iwu, 1985). Also, kolaviron at 100mg/kg body weight reduced thiopental induced sleep CCl4-poisoned rats and protected microsomal enzymes against phalloidin (Iwu *et al*., 1985).

* **Antibiotic Effects**

Aqueous and alcohol extracts of *Garciniakola* was found to inhibit organisms like staphylococcus aureus, klebsiella pneumonia, beta-hemolytic streptococci, escherichia coli and neisseria gonorrhea (Ebana *et al*., 1991).*Garciniakola* extract was also demonstrated to be effective against staphylococcus aureus, streptococcus pneumonia, and hemophilius influenza at a minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) (Akochere *et al*., 2002).Other researchers that observed antibacterial activities are Hussain*et al*, (1982) and Han *et al*., (2005) who observed that *Garciniakola* root extract is also effective against methicillin resistant staphylococcus aureus (MRSA) and vacomycin resistant escherishia coli (VRE). Others are Adeboye *et al*., 2008 and Sibanda and Okoh., 2008.

**(h) Blood Pressure Reducing Effect**

A study carried out with 15 wister rats showed that alcohol extract of *Garciniakola* contains vaso-active substance that has a hypotensive effect. The mechanism of action was not determined but there are indications of possible mediation through histerminergic receptors (Ugwu and Naiho, 2003)

* **Anti-Ulcer Effect**

*Garciniakola* has shown protective effect against HCl and ethanol induced gastric ulcer in rats. Pretreatment of animals with kolaviron at 100mg/kg, orally once a day, reduced formation of ulcers induced by an HCl/ethanol mixture. Gastric juice volume and acidity were also reduced (Olaleye and Farombi, 2002).

Antiulcer activity was also observed against induced gastric mucosal injury in rats with pretreatment of *Garcinia kola* extract that decreased volume and acidity of gastric juice (Mahendran *et al.*, 2002). A similar study in rats found activity against indomethacin-induced gastric ulcers (Mahendran *et al*., 2002).

**(j) Anti-Malaria Effect**

Twenty eight extract from *Garcinia kola* and eight other African medicinal plants used in Congolese traditional treatment for malaria were subjected to pharmacological test to evaluate their effect. Extract of *Garciniakola* stem bark and seed produced 60% inhibition of the growth of plasmodium falciparium invitro at a test concentration of 6microgram/ml (Tona *et al*., 1999).

**(k) Other Observations**

In addition to the above observations, *Garciniakola* extract has been found to have aphrodisiac properties (Ajibola and Satake, 1992). It has also been proved effective in the treatment of dermatological disorders associated with melanin pigmentation (Okunji *et al.*, 2007).

The immunomodulatory and immunorestorative properties of *Garcinia kola* seed extract has been established (Nworu *et al*., 2007; 2008). A study has also shown that *Garciniakola* is a potential osteoarthritis disease modifier with good mid-term outcome (Adegbehingbe *et al*., 2008) *Garciniakola* ophthalmic solution (*Garcinia kola* 0.5% aqueous solution eye drop) significantly reduced intra ocular pressure (IOP) as compared to baseline in patients with Open-Angle Glaucoma or Occular hypertention (Adebununola *et al*, 2010).

If it proves successful in animal and human, it will be the first medication to successfully treat Ebola hemorrhagic fever (an often fatal condition caused by Ebola virus which *Garciniakola* extract was able stop in the laboratory). This discovery was announced at the 16th International Botanical congress in St. Lious in the US by Prof. Maurice Iwu of Nigeria (Iwu, 1999)..

**CHAPTER THREE**

**MATERIALS AND METHODS**

**3.1 PLANT SAMPLE COLLECTION AND EXTRACTION**

Fresh seeds of *Garciniakola* were obtained from local famers in Nsukka Enugu state. Authentication of the seeds was done by Mr. A. O. Ozioko of the Bioresources Development and Conservation Programme Center (BDCP), Nsukka, Enugu State, Nigeria. 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.

**3.2 EXPERIMENTALANIMALS**

Forty five (45) healthy adult nulliparous female Wistar 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.

**3.3 INDUCTION OF PREGNANCY**

Forty (40) of the female wistar rats were randomly selectedand 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.

**3.4 INDUCTION OF DIABETES**

Diabetes was induced in twenty (20) female wistar 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 of 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.

**3.5ACUTE TOXICITY (LD50) TEST OF THE EXTRACT**

This study was conducted according to the Organization for Economic Cooperation andDevelopment (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 using5 healthy adult nulliparous female wistar 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 LD50 is predicted to be above 2000 mg/kg body weight if three or more rats survived.

**3.6 ANIMAL GROUPING AND TREATMENT**

The twenty (20) pregnant but non diabetic rats were randomly assigned into four (4) groups of five (5) rats each and labeled as follows:

* Non-diabetic pregnant control group – Given 0.25 ml of normal saline as single dose daily.

2. Non-diabetic pregnant test groups 1– Given extract orally at 100mg/kg of body weight as a single dose daily.

3. Non-diabetic pregnant test groups 2 – Given extract orally at 200mg/kg of body weight as a single dose daily.

4. Non-diabetic pregnant test groups 3– Given extract orally at 300mg/kg of body weight as a single dose daily.

* The twenty (20) pregnant and diabetic rats were also randomly assigned into four (4) groups of five (5) rats each and labeled as follows:

1. Diabetic pregnant control group – Given 0.25 ml of normal saline as single dose daily.

2. Diabetic pregnant test groups– Given extract orally at 100mg/kg of body weight as a single dose daily.

3. Diabetic pregnant test groups– Given extract orally at 200mg/kg of body weight as a single dose daily.

4. Diabetic pregnant test groups– Given extract orally at 300mg/kg of body weight as a single dose daily.

**3.7 ADMINISTRATION OF EXTRACT**

The method of administration adopted was Oro-gastric intubation using a cannula.

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.

**3.8 SELECTION OF THE OFFSRINGS**

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).

**3.9 SAMPLE COLLECTION AND ANALYSIS**

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 andthen 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.

**CHAPTER FOUR**

**RESULTS**

**4.1: The result of acute toxicity (LD50) test**

The animals were generally dull and still or 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 oftoxicity at the limit dose of 2000mg/kg/oral in thefirst 48 hours and no evidence of toxicity was notedduring the period of observation. The LD50of aqueous extract of *Garcinia kola* seed in ratswas therefore taken as above 2000mg/kg/oral.

Table 4.1: The result of acute toxicity (LD50) test

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

**4.2: The general physical observations on the non-diabetic and diabetic pregnant rats used in the study and their offsprings.**

Table 4.2 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 4.2: Physical observations on the non-diabetic and diabetic pregnant rats used in the study and their offsprings.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Non-diabetic Rats** | | | | | |
| **Groups** | **Number of rats that delivered [n (%)]** | **Total litter size**  **[n]** | **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 |
| **Diabetic Rats** | | | | | |
| **Groups** | **Number of rats that delivered [n (%)]** | **Total litter size**  **[n]** | **Total stillbirths [n(%)]** | **Perinatal death in**  **offsprings [n (%)]** | **Physical anomally**  **in offsprings** |
| **Diabetes 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 |

**CHAPTER FIVE**

**DISCUSSION AND CONCLUSION**

**5.1**

Diabetes mellitus in pregnancy affect the offsprings adversely in utero leadind to leads to complications like miscariage, still births, congenital malformations, etc. (Melamed and Hod, 2009). This is believed to occur because maternal hyperglycemia causes an increased glucose supply to the offspring (Maayan-Metzger *et al*, 2009), leading to fetal hyperglycemia and hyperinsulinemia which promote nutrient storage and macrosomia. The fetal oxygen is used up on this process, leading to hypoxia which in turns leads to oxidative stress and altered antioxidant activities, reduced ascorbic acid levels, and the production of highly reactive oxygen radicals that are toxic especially to cell membranes.

Diabetes mellitus in pregnancy also predisposes the offsprings to increased risk for obesity, glucose intolerance, and diabetes in childhood and or later in life (Buzinaro *et al*, 2008; Simeoni and Barker, 2009).This may be due to the concept of metabolic memory according to Franke *et al* (2005) who observed that diabetic pregnancy causes a defective programming of hypothalamic neurons causing excessive Neuropeptide-Y neurons expression. This they suggestedmay lead to a tendency to overfeeding, overweight and diabetogenic status in the offsprings.

The chances of reducing this poor outcome of pregnancy among diabetics are intricately related with the maintenance of a near normal levelof blood glucose concentration (Shefali *et al*., 2006). 61

**5.2: The general observations during the study.**

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. This hyperglycemia is believed to results from selective necrosis of ß-cells of the islets of Langerhan in the pancreas (Goldner and Gomori, 1944).

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)

**5.3 The effect of *Garciniakola* extract on the weight of non-diabetic and diabetic pregnant rats**

In this study, *Garciniakola* extract significantly reduced the pregnancy induced weight gain among the non-diabetic pregnant rats in a dose dependent manner (Table 4.3a).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 4.3b). 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 4.2).This may be due to the insulinogenic effect of kolaviron an active principle in *Garciniakola* which is believedto possess pancreatic beta cells re-generating, insulin releasing effects and fighting the problem of insulin resistance (Welihinda *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.

**5.4 The effect of *Garciniakola* extract on the fasting blood glucose of**

**non-diabetic and diabetic pregnant rats**

There was a significantly higher fasting blood glucose level (hyperglycemia) among the rats that received an intraperitoneal injection of 80mg/kg of body weight of alloxan hydrate. This supports the existing literature Goldner and Gomori, (1944) demonstrated that alloxan hydrate causes insulin dependent diabetes in rodents by the necrosis of the islets of Langerhans.

*Garcinia kola* extract significantly reduced diabetes induced hyperglycemia as the pregnancy advanced in a dose dependent manner (Table 4.4b).There is paucity of data on the anti diabetic activities of the extract in pregnancy, but this supports the glucose lowering activities of *Garciniakola* as observed by previous studies (Iwu *et al*, 1990; Adaramoye and Adeyemi, 2006).

This may be due to the insulinogenic effect of kolaviron an active principle in *Garciniakola* which is believedto possess pancreatic beta cells re-generating, insulin releasing effects and fighting the problem of insulin resistance (Welihinda *et al*, 1982; Hongxiang *et al*, 2009). 63

**5.5 The effect of *Garciniakola* extract on litter size of non-diabetic and diabetic pregnant rats**

The result of this study indicates that diabetes in pregnancy significantly reduced the litter size in wistar albino rats (Table 4.5b).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.

*Garciniakola* extract significantly increased the litter size among the diabetic pregnant rats in a dose-dependent manner in this study (Table 4.5b).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. 64

**5.6 The effect of *Garciniakola* extract on the weight of the offsprings of**

**non-diabetic and diabetic pregnant rats**

Macrosomic (large) babies were observed among the litters of the rats in diabetic control group in this study (Table 4.6b). These large babies maintained an accelerated weight gain even up to day 21 (Table 4.6b). and this is in line with the existing literature that maternal hyperglycemia leads to excessive glucose delivery during diabetic pregnancy, leading excessive growth of the offspring which often extends into childhood and even adult life (Yessoufou, 2006; Merzouk *et al*, 2002).

The extract caused significant reduction in the sizes of the offsprings in a dose-dependent manner in this study in the diabetic test groups (Table 4.6b).

This may be as a result of the glucose lowering activities of the *Garciniakola* extract in the mother which leaves little or no excess glucose for the baby to absorb and become macrosomic.

**5.7 The effect of *Garciniakola* extract on fasting blood glucose level of the offsprings of non-diabetic and diabetic pregnant rats on the weaning (21st) day**

This study also showed a significantly higher fasting blood glucose level among offsprings of the diabetic mothers on the weaning day (Table 4.7b). This is in agreement with the current observation in the literature which suggests that diabetes in pregnancy predisposes the offsprings of the pregnancy to diabetes in childhood or adolescence 65

(Pontiroli, 2004), and that fetuses of diabetic women that are born large for gestational age appear to be at the greatest risk (Eriksson *et al*, 2003).

The extract caused a statistically significant dose-dependent reduction (p<0.05) in the fasting blood glucose profile among the offsprings of the diabetic mothers (Table 4.7b). This may also be due to the glucose lowering activities of the *Garciniakola* extract in the mother which leaves little or no excess glucose for the offsprings to absorb to become hyperglycemic and hyperinsulinemic that will predispose them to glucose intolerance and childhood diabetes.

In summary, the poor outcome of diabetic pregnancies and the effects they have on the offspring later in life are consequences of maternal hyperglycemia. Both the oxidative stress and in-utero defective programming of the hypothalamic neuropeptide –Y neurons that are believed to be the cause of these complications are results of maternal hyperglycemia. That is to say that, reducing the poor outcome of diabetic pregnancy is strongly tied to glycemic control in pregnancy (Shefali *et al*., 2006; Kingsley, 2007) which the *Garciniakola* extract was able to do (Table 4.4b).

**5.2 CONCLUSION**

The observations of this study suggest that *Garciniakola* may serve as a potent hypoglycemic agent for the management of diabetes in pregnancy.This study also suggests that *Garciniakola* can reverse the adverse effect of diabetes in pregnancy on both the mother and the offspring in childhood.

**RECOMENDATIONS**

Following the findings of this study, it is recommended that further studies of longer duration be carried out to see to see the effect of this extract on the offsprings latter in life.

Itis also recommended that another study be carried out to isolate the active principle of *Garcinia kola* responsible for the observed effects so as to elucidate the mechanism or pathway of action.

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