

Cellular Mechanisms of Saline Extract of Alligator Pepper (*Zingiberaceae Aframomum melegueta*) for Specific Protection against Fetal Macrosomia

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Abstract: Introduction: Aqueous extract of Alligator pepper is known to reduce gestational weight gain and litter size in Sprague Dawley rats. This study was done to determine the underlying hormonal and cellular mechanisms of action.

Methods: We performed a controlled cross sectional intervention study with 45 female Sprague Dawley rats, which after acclimatization for two weeks, were allowed to mate with male rats for three days. Thereafter, these female rats were randomly allocated into three groups A, B and C with 15 female rats in each group. These three major groups were further randomly allocated into three subgroups so that each subgroup had 5 female rats. Pregnant female rats in Group A were injected with 2ml of normal saline intraperitoneally on day 4. Pregnant female rats in groups B and C were injected intraperitoneally with 6.7mg/Kg body weight and 13.3mg/ kg body weight of saline extract of Alligator Pepper respectively on day 4. Glucose levels were estimated with glucometer on days 7, 14, and 21. Blood was collected by intra-ventricular puncture and assayed for insulin levels. Observed differences between control and experimental groups were subjected to tests of significance.

Results: Alligator pepper treated pregnant rats had significantly higher serum glucose levels than control group. Low dose and high dose Alligator pepper depressed serum insulin levels in the experimental group on day 7 and days 7 and 14 respectively

Conclusion: Intraperitoneal injection of saline extract of Alligator pepper prevents first and second trimester hyper-insulinemia in pregnant Sprague Dawley rats.

Keywords: Cellular Mechanisms, Alligator pepper, Specific Protection, Fetal Macrosomia.

INTRODUCTION

In a study of gestational weight gain and pregnancy outcome in 481 glucose tolerant obese women, it was found that increasing weight gain in obese women was associated with increasing pregnancy complications and it was suggested that minimal gestational weight gain might normalize birth weight [1]. In a five year study in Florida involving 660,396 pregnant women from different racial and ethnic groups, overweight and obesity, excessive gestational weight gain, and gestational diabetes mellitus were associated with large for gestational age babies. However, preventing excessive gestational weight gain had the greatest potential to reduce the risk of fetal macrosomia [2]. Since aqueous extract is known to reduce gestational weight gain and litter size in Sprague Dawley rats without morphologic or genetic defects in the offspring, [3] this study was done to determine the underlying hormonal and cellular mechanisms of action of Alligator pepper (*Zingiberaceae aframomum melegueta*).

METHODS

We conducted a controlled cross sectional intervention study. After acclimatization for two weeks in cages, during which all rats were fed with water and grower's mash *ad libitum*, 45 male and 45 female Sprague Dawley rats were allocated to cages in such a way that each cage contained one male and one female rat. They remained in these cages for three days during which they were expected to mate. Thereafter, the female rats were randomly allocated into three groups A, B and C with 15 female rats in each group. These three major groups were further randomly allocated into three subgroups of 5 female rats each so that these sub groups were then labeled A₁, A₂ A₃; B₁, B₂, B₃; and C₁, C₂, C₃.

Pregnant female rats in Group A were injected with 2ml of normal saline intra-peritoneally on day 4. Pregnant female rats in groups B and C were injected intraperitoneally with 6.7mg/Kg body weight and 13.3mg/ kg body weight of saline extract of Alligator Pepper respectively on day 4 in line with the doses used in an experiment on the effect of aqueous extract of Alligator pepper on gestational weight gain by

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Inegbenebor *et al.*, in 2009. The dose of 6.7Kg /body weight of Alligator pepper was christened 'low dose' Alligator pepper extract while the dose of 13.3 kg/ kg body weight of Alligator pepper was christened 'high dose' Alligator pepper extract in order to distinguish between the two doses nominally and in ranking.

All rats in subgroups A₁, B₁, C₁; A₂, B₂, C₂; and A₃, B₃, C₃ were euthanized by cervical dislocation on days 7, 14 and 21 respectively following chloroform anesthesia. Blood was collected by intra-ventricular aspiration and assayed for insulin levels using Insulin ELISA (Ref EIA 2935) produced by DRG Instruments GmbH, Marburg, Germany. Pregnancy was confirmed by dissecting the uteri of the rats to reveal the embryo/fetuses in their gestational uterine cavity. Glucose levels were estimated from capillary blood from the rats' tail after a 12 hour fast using a

glucometer (ACCU CHEK; Roche.) on days 7, 14, and 21. Means and standard deviations were calculated for each of the subgroups and observed differences between control and experimental groups were subjected to tests of significance.

RESULTS

Serum fasting glucose levels were significantly higher in Alligator pepper treated rats on days 7, 14 and 21. (P= 0.000, 0.000, 0.001 on days 7, 14 and 21 respectively) Higher dose of Alligator pepper only made a significant difference in increasing serum fasting glucose level on days 7 and 21. (P= 0.004 and 0.000 on days 7 and 21 respectively) See Table 1.

Low dose Alligator pepper treated pregnant rats had lower serum insulin levels than control groups on day 7 only while higher dose Alligator pepper treated rats had

Table 1: Time and dose Effect of Alligator Pepper on Serum Glucose Level in Pregnant Sprague Dawley Rats

Group		No of Rats	Pregnancy Status	Dose of Alligator Pepper in Kg/body wt	Mean Blood Glucose Level±SD mmol/l		
					Day 7	Day 14	Day 21
A	Control	15	Pregnant	Nil	4.85±0.62 (Subgrp A ₁)	4.22±0.23 (Subgrp A ₂)	3.69±0.16 (Subgrp A ₃)
B	Experimental	15	Pregnant	6.7mg	6.53±0.23 (Subgrp B ₁)	5.55±0.45 (Subgrp B ₂)	5.03±0.55 (Subgrp B ₃)
P-value (A and B)					0.000**	0.000**	0.001**
C	Experimental	15	Pregnant	13.3mg	6.06±0.13 (Subgrp C ₁)	5.89±0.25 (Subgrp C ₂)	7.5±0.07 (Subgrp C ₃)
P value (A and C)					0.003**	0.000**	0.000**
P value (B and C)					0.004**	0.178	0.000**

**Significant.

Table 2: Time and dose Effect of Alligator Pepper on Blood Insulin in Pregnant Sprague Dawley Rats

Group		No of Rats	Pregnancy Status	Dose of Alligator Pepper in Kg/body wt	Mean Blood Insulin Level±SD microinternational units/ml		
					Day 7	Day 14	Day 21
A		15	Pregnant	-	3.08±2.3 (Subgrp A ₁)	1.95±1.92 (Subgrp A ₂)	0.80±0.23 (Subgrp A ₃)
B	Experimental	15	Pregnant	6.7mg	0.7±0.12 (Subgrp B ₁)	2.3±1.73 (Subgrp B ₂)	1.35±0.17 (Subgrp B ₃)
P value (A and B)					0.05	0.770	0.003**
C	Experimental	15	Pregnant	13.3mg	1.45±0.06 (Subgrp C ₁)	0.85±0.40 (Subgrp C ₂)	1.25±0.06 (Subgrp C ₃)
P value (A and C)					0.152	0.245	0.003**
P value (B and C)					0.000**	0.105	0.250

**Significant.

lower insulin levels days 7 and 14 See Table 2. While the extract of Alligator pepper caused a reduction in serum insulin levels, there was a significant increase in fasting blood glucose levels.

DISCUSSION

The affluent woman, who is pampered with delicacies such as high energy drinks and food found in fast food restaurants or the rural or urban poor who eats large quantities of high glycemic index diets like cooked fermented cassava [4] is exposed to high level of serum glucose. This tends to build fat in the presence of high level of insulin secreted in response to high blood glucose levels [5]. This may lead to obesity, insulin resistance and sometimes hypertension [6, 7]. Such women are predisposed to being pregnant with large for gestational age babies (fetal macrosomia) [2].

The weight of a child at birth is a critical determinant of neonatal morbidity and mortality and the risk of metabolic disease throughout the life span. Growth retarded infants, such as those with intrauterine growth retardation and those that are small for gestational age, are predisposed to hypoglycemia, hypocalcemia, hypothermia, asphyxia, and cognitive dysfunction while those who have rapid catch-up weight gain in early childhood are at increased risk for adult-onset type 2 diabetes, hypertension, preeclampsia, dys-lipidemia, and ischemic heart disease [8]. Likewise, large for gestational age infants (fetal macrosomia) are predisposed to childhood weight gain, type 2 diabetes, and the metabolic syndrome [9].

The outcome of pregnancy is related to the physiological and metabolic changes in pregnancy [10]. Serum insulin levels determine the utilization of nutrients in the growth and development of the mother and fetus. Furthermore, cellular resistance to insulin may amplify the effect of metabolites on the growth and development of the fetus [7]. It may also be responsible for gestational diabetes mellitus and preeclampsia, which may be adverse to pregnancy outcome [6]. An important adverse pregnancy outcome is fetal macrosomia

In a previous study it was found that intraperitoneally injected extract of Alligator pepper was capable of reducing gestational weight gain and litter size [3].

Since fetal macrosomia is related to pre-gravid obesity and gestational weight gain even in glucose

tolerant women [1], gestational weight gain reduction may be capable of improving pregnancy outcome through the prevention of fetal macrosomia and obviation of the need for risk laden obstetric interventions.

In a five year study in Florida involving 660,396 pregnant women from different racial and ethnic groups, overweight and obesity, excessive gestational weight gain, and gestational diabetes mellitus were associated with large for gestational age babies. However, preventing excessive gestational weight gain had the greatest potential to reduce the risk of large for gestational age babies or fetal macrosomia [2].

This study was therefore carried out to determine the changes in the levels of serum glucose and insulin with the aim of understanding the physiological mechanisms behind the effects of aqueous extract of Alligator pepper on gestational weight gain and litter size.

Administration of low dose saline extract (6.7/kg body weight) of Alligator pepper caused a significant increase in glucose level (See Table 1) and non significant decrease in insulin level on day 7 of pregnancy (See Table 2). The main function of insulin in the body is to store nutrients either as glycogen in the liver and muscles or as fat in the adipose tissue. The quantity and quality of storage that occurs depends on the type of diet and the time of consumption. When a large quantity of high glycemic index carbohydrate meal is consumed, a large amount of glucose is released post digestion into the blood stream. This stimulates insulin response, which converts it into glycogen initially. If the glucose is excessive or available when the individual is not exercising, it is converted into fat in the adipose tissue [11]. In addition, large insulin response inhibits the release of leptin, which is supposed to activate the satiety center. Consequently, the individual gets the feeling that he is not satisfied. This leads to a craving for more glucose producing diets, increasing fat storage and finally obesity [11]. Pregnant women are not spared in this phenomenon. This may lead to excessive gestational weight gain and fetal macrosomia. In this study, intraperitoneal injection of low dose (6.7mg/Kg body weight) of Alligator pepper decreased serum insulin levels on day 7 of pregnancy (See Table 2), while a higher dose 13.3mg/Kg body weight) decreased insulin levels on days 7 and 14 of pregnancy (See Table 2). Depressed insulin level may reduce body fat in various ways. First, it is unable to

convert all the glucose released into the blood into fat. Secondly it is unable to inhibit leptin and therefore satiety is guaranteed after feeding, so that the animal takes what it needs for maintaining the metabolic process only [11]. Thirdly, it is unable to produce adequate amount of alpha glycerophosphate, an end product of insulin enhanced glucose metabolism, which is necessary for formation of fat [12] and the individual does not get overweight. In addition, the beta and trans forms of caryophyllene, a constituent of Alligator pepper [13] activates type 2 cannabinol receptor (CB2R) selectively and accelerates lipid oxidation, which results in weight loss [14].

It is pertinent at this juncture to explain the probable mechanism by which the extract of Alligator pepper reduces serum insulin. Major constituents of Alligator pepper, trans caryophyllene and alpha-humulene (sesquiterpenes) [13], which reduce the secretion of tumor necrotic factor alpha [15, 16]. Tumor Necrotic factor alpha is known to reduce glucose-stimulated insulin secretion in pancreatic beta-cells [17]. Trans-caryophyllene also modulates glucose stimulated insulin release [18].

Unlike what has been found in previous study by Mojekwu and other researchers [19], there was a significant rise in blood glucose rather than a decrease (See Table 1). Mojekwu and colleagues [19] used the blood obtained from intra-ventricular puncture in their study while capillary blood from the rat's tail was used in this study after a 12 hour fast. Capillary blood in the rat is known to have lower glucose levels than the blood from the ventricles or aorta [20]. Yet the glucose levels in this study are higher than those obtained in Mojekwu's study. This discrepancy may depend on the glycemic load (Glycemic index and quantity) of carbohydrate content of rat feed used in both experiments. The level of fasting blood glucose in the rats treated with low dose Alligator pepper extract was outside the normal range in the first trimester only while the fasting blood glucose level in the rats treated with high dose Alligator pepper extract fell outside the normal range in the third trimester only, according to the range determined by Wang and colleagues in 2010 [20]. In a study to estimate serum blood glucose in rats, Wang *et al.* found that the average fasting blood glucose and post prandial blood glucose of normal Wistar rats was (3.95 +/- 1.31) mmol/L and (5.65 +/- 1.63) mmol/L, and the 95% upper limit was 6.2 mmol/L and 7.9 mmol/L respectively. Blood glucose from abdominal aorta was 40% higher than that from tail. After the test requirement being regulated, the upper

limit for Fasting Blood Glucose and Post prandial Blood Glucose was expanded to 7.5 mmol/L and 10.4 mmol/L, respectively. They concluded that serum blood glucose levels in rats are similar to those of humans [20].

Normal values of fasting blood glucose in human pregnancy seem to vary from one race to the other. Normal values of fasting blood glucose have been stated as 83.5-85.75mg/dl, (4.64-4.76mmol/l), 81.95-83.45mg/dl, (4.55-4.64mmol/l) 84.3-86.5 mg /dl (4.68-4.81mmol/l) for first, second and third trimesters respectively for pregnant women in Saudi Arabia [21]; 70.75mg/dl,(3.93mmol/l) 66.10mg/dl (3.67mmol/l) and 66.80mg/dl (3.71mmol/l) for first, second and third trimesters respectively in Nigeria [22] and 78mg/dl ((4.33mmol/l) and 76mg /dl (4.22mmol/l) for first and third trimesters respectively in Israel [23]. This may be related to dietary habits of women of various races.

Serum insulin level of rats treated with low and high doses of Alligator pepper extract were lower than the corresponding levels in the first trimester (day 7) and first (day 7) and second (day 14) trimesters of pregnancy respectively. This can be described as depression of insulin secretion from the pancreas, which may be interpreted as attenuation of the secretion from the beta cells of the pancreas or an increase in insulin sensitivity due to up-regulation of the insulin receptors. However, the actual effect cannot be determined within the scope of this study as the quantity of receptors could not be estimated. An increase in insulin sensitivity or reduction of insulin secretion can be interpreted as reduction of the insulin resistance in pregnancy and an implication that both body weights of the mother and fetus will be reduced [11].

CONCLUSION

Intraperitoneal injection of saline extract of Alligator pepper causes gestational weight gain reduction, litter size reduction [3], by preventing hyper-insulinemia in pregnant Sprague Dawley rats in the first and probably second trimesters of pregnancy.

RECOMMENDATION

We suggest that a vaccine that depresses serum insulin level through some of the several mechanisms of action discussed in this article should be developed from the active constituents of Alligator pepper or any other substance for specific protection against fetal macrosomia.

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CONFLICT OF INTEREST

None declared.

AUTHOR'S CONTRIBUTION

U.I carried out the research and also wrote the article under the supervision of M.E.

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