

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

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Abstract: Preeclampsia is a clinical syndrome defined as the new onset of hypertension and proteinuria during the second half of pregnancy. Though it is easily diagnosed clinically, affected persons must book in a health service facility for the diagnosis to be made. Furthermore regular screening is necessary during several antenatal visits and skilled attendant's supervised labor before diagnosis can be made. Predictive tests are not yet well developed and not readily available in developing countries and medically underserved areas where they are needed most. There is therefore a need for specific protection against preeclampsia to be developed. This will ensure that all women are protected even when they choose not to visit an antenatal clinic. Specific protection is potentially capable of preventing preeclampsia in 5 % to 7% of pregnant women, the proportion of pregnant women that are affected by preeclampsia worldwide. This article builds on a previous article on the study of the cellular mechanisms underlying gestational weight gain and litter weight reduction effect of aqueous extract of Alligator pepper and attempts to analyze how the anti-hyperinsulinemic property of the constituents of Alligator pepper in that study can also prevent preeclampsia and suggests the use of some of the constituents of alligator pepper as specific protection (vaccine) for the prevention of preeclampsia.

Keywords: Cellular Mechanisms, Alligator pepper, Preeclampsia, Specific protection.

INTRODUCTION

Preeclampsia is a clinical syndrome defined as the new onset of hypertension and proteinuria during the second half of pregnancy [1]. Both hypertension and proteinuria implicate the endothelium as the target of the disease [2]. Common risk factors include first pregnancy, new paternity, teenage pregnancy, pregnancy in the forties, prolonged interval between pregnancies, diabetes mellitus in pregnancy, and chronic vascular hypertensive disease [3]. Common complications include stroke, placental abruption, seizures, low fetal birth weight and hemorrhage. Once preeclampsia is present in a pregnancy, there is no definitive cure other than to deliver the child and the placenta. Preeclampsia carries with it a significant risk of injury or death to both the mother and child [4].

The ability to predict preeclampsia is currently of limited benefit because, neither the development of the disorder, nor its progression from the mild to the severe spectrum of disease, can be prevented in most patients. Besides, there is no cure except delivery [5]. Up till recently there were no clinically available tests that perform well in distinguishing women who will

develop preeclampsia from those who will not [6]. Researchers have now found that among pregnant women who developed preeclampsia later in pregnancy, the number of capillaries was reduced early in pregnancy—even at 20 weeks of pregnancy. Data show that using this novel, simple measure seemed to identify those women who are likely to develop preeclampsia 87 percent of the time—an improvement on the currently used screening test [7]. Repeating the test during the course of pregnancy at or after 27 weeks gestation accurately identified the condition 75 percent of the time and was less likely to include false positives - women who would not go on to develop the condition. However most of the predictive tests are not yet available in most developing countries where clinical diagnosis can only be made during antenatal screening of patients and sometimes for the first time in labor. It is therefore obvious that in medically underserved areas and places where there is low patronage of antenatal clinics, several women will develop preeclampsia and only present in labor with eclamptic fits in traditional birth attendant's homes or as unbooked patients in health care providers' clinics that are least prepared for the much needed attention that eclampsia deserves. It is therefore necessary to develop a method of preventing the development of the disease. Since there are no definite (health education and promotion) methods of averting the development of the disease and early diagnosis and treatment are only

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possible where women turn up in antenatal clinics for screening, specific protection becomes the most important tool that can be used for mass protection of women from this second leading cause of maternal mortality, worldwide behind complications of child birth itself [7].

This article is a build up on a controlled cross sectional intervention study to determine the cellular mechanisms underlying the gestational weight gain and litter size reduction effects of intraperitoneally injected saline extract of Alligator pepper (*Zingiberaceae Aframomum melegueta*) [8] described below for easy reference.

RESEARCH 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 the 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 Inegbenebor and colleagues in 2009 [9]. The dose of 6.7mg/Kg body weight of Alligator pepper was christened 'low dose' Alligator pepper extract while the dose of 13.3mg / 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 [8].

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 [8].

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

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.

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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	Control	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.

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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 [8].

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 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 [8].

DISCUSSION

Insulin levels determine the utilization of nutrients in the growth and development of the mother and fetus. However, cellular resistance to insulin may amplify the effect of metabolites on the growth and development of the fetus [10]. It may also be responsible for gestational diabetes mellitus and pregnancy induced hypertension, which may be adverse to pregnancy outcome [11]. In multivariate analyses of 481 glucose tolerant obese women, increasing weight gain was associated with significantly higher rates of hypertension (OR 4.8 [95% CI for group 4 vs. group 1: 1.7-13.1]), cesarean section (3.5 [1.6-7.8]), induction of labor (3.7 [1.7-8.0]), and large-for-gestational-age infants (4.7 [2.0-11.0]) [12].

Several authors have attempted to link preeclampsia with hyperinsulinemia. In a controlled study to assess whether hyperinsulinemia is present in hypertensive disease induced by pregnancy, It was concluded that preeclampsia is associated with marked

hyperinsulinemia both in the fasting state and after oral glucose ingestion, suggesting that insulin resistance may play a role in pregnancy-induced hypertension [13]. In another case control study of fasting insulin levels in normal pregnancy and pre-eclampsia patients, it was concluded that an exaggeration of the insulin resistance is seen with the resultant hyperinsulinemia in women with pregnancy complicated by preeclampsia. The results of the study also showed that hyperinsulinemia was directly proportional to the severity of the disease [14]. Aside from insulin resistance, endothelial activation and low grade inflammation play an integral role in the preeclampsia/eclampsia, obesity and gestational diabetes. Insulin resistance with secondary hyperinsulinemia is suspected to be the link between hypertension and diabetes mellitus. The hypertensive effect of hyperinsulinemia is postulated to be due to weight gain as well as extracellular volume expansion. This volume expansion is thought to result from renal sodium retention caused by insulins effect on sympathetic activity [15].

In a previous study, it was found that intra-peritoneally injected extract of Alligator pepper was capable of reducing gestational weight gain and litter size [9]. In an attempt to study the cellular mechanisms underlying the effects of Alligator pepper extract, it was found that saline extract of Alligator pepper depresses blood insulin level [8]. Hyperinsulinemia of pregnancy is related etiologically to the development of preeclampsia and the onset of preeclampsia is usually in the second or third trimester of pregnancy [13-15]. Since the saline extract of Alligator pepper reduced

fasting blood insulin levels on day 7 (end of first trimester) at the dose of 6.7mg/Kg body weight and also reduced fasting blood insulin levels on days 7 and 14 (end of first and second trimester) at a higher dose of 13.3mg/Kg body weight, we are postulating that the saline extract of Alligator pepper can prevent the development of preeclampsia (See Table 2).

Certain sesquiterpene constituents of Alligator pepper, namely, trans-caryophyllene and alpha-humulene [16] reduce the secretion of tumor necrotic factor alpha [17, 18]. Tumor Necrotic factor alpha is known to reduce glucose-stimulated insulin secretion in pancreatic beta-cells [19]. Trans-caryophyllene also modulates glucose stimulated insulin.

High insulin level causes hypertension by increasing sodium ion retention through a direct increase on the activity of the epithelial sodium channel (ENaC), the sodium phosphate co-transporter, sodium hydrogen exchanger type III and Na-K-ATPase in the kidney [20]. Insulin also induces oxidative stress, which causes impairment of the vascular endothelium, decreased availability of nitric oxide, and therefore atherosclerosis and hypertension [21]. Insulin causes excitation of the sympathetic nervous system *via* a central action on the hypothalamus, where it is believed to act on the anteroventral third ventricle hypothalamic region [22]. Other researchers have found that hyperinsulinemia in normotensive humans increases sympathetic nerve activity but not arterial pressure since it also causes skeletal muscle vasodilatation. However, in the presence of insulin resistance and/or hypertension, insulin may cause exaggerated sympathetic activation or impaired vasodilatation and thus elevate arterial pressure [23]. In addition, high level of insulin as seen in insulin resistance suppresses intracellular magnesium. The suppressed intracellular magnesium concentration may result in defective tyrosine kinase activity and modify insulin sensitivity by influencing receptor activity after binding or by influencing intracellular signaling and processing.

Intracellular magnesium deficiency may affect the development of insulin resistance and alter the glucose entry into the cell. Magnesium is therefore required for both proper glucose utilization and insulin signaling. Metabolic alterations in cellular magnesium, which may play the role of a second messenger for insulin action, contribute to insulin resistance [24]. The reduced intracellular magnesium causes an increase in vascular tone and therefore hypertension [25]. Low insulin levels as produced by Alligator pepper extract may therefore

be capable of increasing intracellular magnesium and reducing vascular tone in pregnancy. This effect may therefore be useful in the prevention of preeclampsia and therefore eclampsia. Reduction of insulin levels by extract of Alligator pepper may also preserve vascular endothelium and the nitric oxide it secretes [21] and therefore prevent pregnancy induced hypertension and eclampsia.

CONCLUSION

We conclude that intraperitoneal injection of saline extract of Alligator pepper may prevent preeclampsia by preventing hyper-insulinemia as has been demonstrated in this study using the pregnant Sprague Dawley rats' model.

RECOMMENDATION

In order to provide mass specific protection for the illiterate, ignorant and poverty stricken women who live in medically underserved areas, we suggest that a vaccine should be developed from Alligator pepper that can act as a specific protection for preeclampsia, a precursor of eclampsia that is the second highest cause of maternal mortality [7].

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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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