

## EFFECT OF AQUEOUS EXTRACT OF ALLIGATOR PEPPER (*ZINGIBERACEA AFRAMOMUM MELEGUETA*) ON LITTER SIZE OF ALLOXAN INDUCED DIABETIC SPRAGUE DAWLEY RATS

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### ABSTRACT

This study was done to find out if aqueous extract of alligator pepper can prevent the development of fetal macrosomia in type 1 diabetic pregnant rats. Eighteen female Sprague Dawley rats were randomly allocated into three groups A, B and C (n=6 each). Type 1 diabetes mellitus was induced in the rats in groups B and C. Seventeen female rats, which met the criteria for inclusion, were mated with 17 male rats in separate cages. Thereafter, the pregnant female rats were placed in separate maternity cages and fed with growers mash and water *ad libitum*. On day 4 of gestation, the rats in groups A and B were given an intra-peritoneal injection of 13.3ml/Kg body weight of distilled water, while the rats in group C were given an intra-peritoneal injection of 13.3mg/Kg body weight of aqueous extract of alligator pepper. There was a non-significant increase in the weight of litters from non-treated diabetic rats in group B ( $p > 0.05$ ) and a significant decrease in the weight of litters of treated diabetic pregnant rats in group C ( $p < 0.05$ ). It was concluded that alligator pepper significantly reduces litter size in pregnant Sprague Dawley rats with type 1 diabetic mellitus.

**Key words:** Effect, Alligator Pepper, Litter Size, Type 1 Diabetes Mellitus, Sprague Dawley Rats

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### INTRODUCTION

Alligator pepper (*Zingiberaceae Aframomum melegueta*) is a widely used spice in several parts of the world. Also known as Grains of paradise, *Aframomum melegueta*, a species of the ginger family, contains pungent, aromatic ketones such as 6-paradol, 6-gingerol and 6-shogaol (Sugita *et al.*, 2013). Previously, two sesquiterpene hydrocarbons, humulene and caryophyllene and their oxides had been identified as constituents that made up 82.6% and 9% respectively of an essential oil, obtained by hydrodistillation, of the seeds of alligator pepper (Ajaiyeoba and Ekundayo, 1999). In a more recent study, Owokotomo *et al.* (2014) identified 9 constituents [mainly  $\alpha$ -caryophyllene (48.78%),  $\beta$ -caryophyllene (32.50%) and linalool (5.40%)] in the seeds of alligator pepper.

On the other hand, fetal macrosomia is a common outcome of pregnancy in women affected by diabetes mellitus, gestational diabetes mellitus, pre-gravid obesity and excessive gestational weight gain (Herring and Oken, 2011). It has several undesirable outcomes of labor; the most enigmatic of which is shoulder dystocia. It also predisposes to childhood obesity with potential implications for adult obesity and the metabolic syndrome (Cornier *et al.*, 2008). Even when blood sugar is controlled in some diabetic pregnant women, a high proportion still develops fetal macrosomia (Keely and Barbour, 2014). In a controlled study of 101 diabetic pregnant women in Australia, glucose control did not appear to have a direct effect on the incidence of macrosomic babies, and it was concluded that such observation might result from the effects of other confounding factors. (Wong *et al.*, 2002).



Recently, the level of maternal triglycerides (TGs) have been strongly correlated with excess fetal growth and fetal macrosomia (Schaefer-Graf *et al.*, 2008), supporting another suggestion that other maternal fuels such as triglycerides and free fatty acids play an important role in excess fetal fat accretion (Keely and Babour, 2014).

Since intra-peritoneal injection of aqueous extract of alligator pepper has been found to reduce litter size of pregnant Sprague Dawley rats without affecting the morphology and genetic or reproductive capability of the off-springs (Inegbenebor *et al.*, 2009), this study was carried out to determine the possibility of its use in preventing the development of fetal macrosomia in type 1 diabetic mothers using pregnant Sprague Dawley rats as the animal model.

## MATERIALS AND METHODS

**Experimental Animals/Acclimatization:** 18 female and 18 male Sprague Dawley rats of proven fertility and weighing between 140-160g were acclimatized in a well-ventilated laboratory for a period of two weeks. During this period, they were placed in cages and fed with growers mash and water *ad libitum*. Their beddings, which were made of saw dust, were changed daily. The animals were weighed weekly throughout the duration of the study.

**Grouping:** The female Sprague Dawley rats were allocated into three groups A, B and C so that each group had 6 rats.

**Induction of Diabetes Mellitus in Groups B and C:** Twelve female rats in groups B and C were fasted overnight and diabetes mellitus was induced by the intra-peritoneal administration of 150mg/Kg body

weight of freshly prepared alloxan normal saline solution as described by Sekar *et al.* (1990). Thereafter, the animals were allowed to drink 5% dextrose in water in order to overcome the drug induced hypoglycemia (Gandhi and Sasikumar, 2012). A period of one week was allowed for the development of diabetes mellitus. Thereafter, rats with blood glucose level greater than 200mg/dl (11.1mmol/l) were considered diabetic. However one of the female rats in group C had a glucose level of 194mg/dl (10.8mmol/l) and was excluded, making only 5 female rats available in group C for the study

**Mating Arrangement:** Seventeen rats, (6 each in groups A and B and 5 in group C), were mated with male rats of proven fertility as monogamous pairs in separate cages for three days. After copulation was confirmed to have occurred, the female rats were placed in separate maternity cages and fed with water and grower's mash *ad libitum* till delivery.

**Measurement of litter weight:** After delivery, the litters from each group were weighed in a top loading balance and mean litter weight was calculated for each of the groups A, B and C.

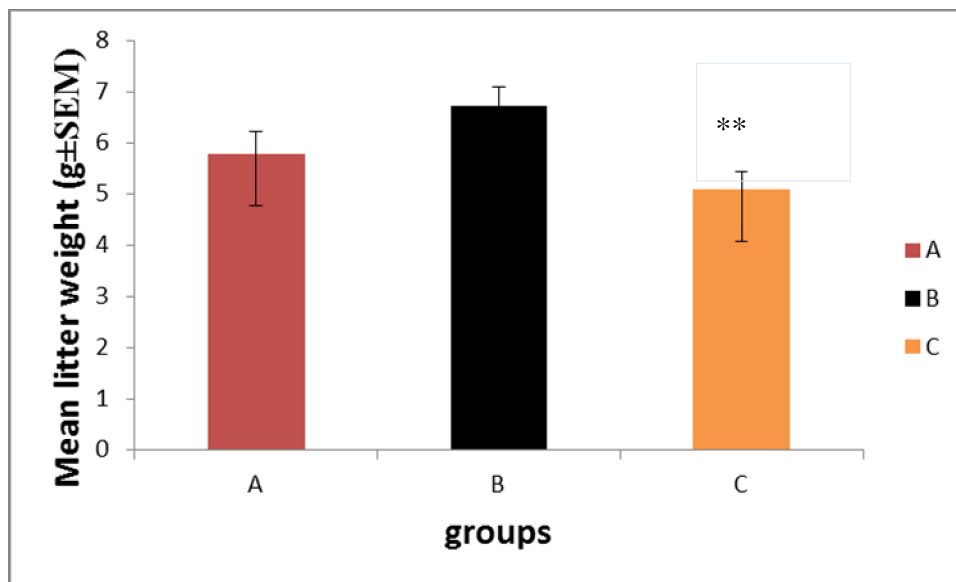
**Data Analysis:** The mean and standard errors of mean of the weights of the litters for the different groups were calculated. Bar charts with error bars were designed to display the observed differences in litter weight. The differences between the mean litter weights of the control and experimental groups were subjected to tests of significance using Daniel Soper's free website for calculating p-values (Soper, 2016). P<0.05 was considered as significant.

**Table 1: Effect of Aqueous extract of Alligator pepper on litter weight of Alloxan-induced Diabetic Rats**

Grps	No of rats	Dose of Alligator Pepper (IP)	Dose of Distilled water (IP)	Status	No of litters	Mean weight of litters ± SEM
A	6	---	13.3ml/Kg	Pregnant; non diabetic	39	5.77±0.46
B	6	---	13.3ml/Kg	Pregnant; diabetic	39	6.72±0.38
C	5	13mg/kg	---	Pregnant; diabetic	29	5.07±0.35**

\*\* Significant decrease in litter weight (p < 0.05); IP = Intraperitoneal





\*\* p < 0.05

**Figure 1: Effect of Aqueous extract of Alligator pepper on litter weight of alloxan induced diabetic rats.**

## DISCUSSION

Fetal overgrowth in pregnancies complicated by diabetes is the result of an increased substrate availability, which stimulates fetal insulin secretion and fetal growth (Jansson *et al.*, 2006). In an earlier study, it was found that intra-peritoneally injected aqueous extract of alligator pepper reduced litter size of pregnant Sprague Dawley rats without adverse effects (Inegbenebor *et al.*, 2009). In a more recent study, it was suggested that the mechanism of action of alligator pepper is the creation of an environment of relative insulin deficiency in the maternal blood, which reduces glucose uptake into the fetal compartment and consequently reduces the quantity of fetal insulin released by the fetal pancreas. The reduced level of fetal insulin is believed to reduce glucose utilization in the fetus, thereby reducing fat and muscle mass and consequently fetal size (Inegbenebor *et al.*, 2016). This explanation may provide adequate explanation if it were ethical to administer intra-peritoneal dose of aqueous extract of alligator pepper to pregnant women who are either obese or have developed type 2 diabetes mellitus. However, the capability of aqueous extract of alligator pepper to reduce litter weight of type 1 diabetic pregnant Sprague Dawley rats, as

observed in this study, where alloxan was used in inducing type 1 diabetes mellitus, implies that there may be other mechanisms of action.

In spite of the insulin deficiency in type 1 diabetes mellitus, affected mothers give birth to macrosomic babies. This is due to the quantity of glucose transferred from maternal blood to the fetus through fetal maternal circulation. This stimulates the fetal pancreas to produce large quantity of insulin, which being a hormone of accretion, increases fat mass, and muscle mass as well as longitudinal bone growth (Jansson *et al.*, 2006). It appears that aqueous extract of alligator pepper is able to reach the fetus through the fetomaternal circulation. This creates a relative insulin deficiency in the fetus as was found in alligator pepper treated pregnant rats in a previous study (Inegbenebor *et al.*, 2016). In the presence of relative insulin deficiency, there is dis-inhibition of triglyceride lipase, which enhances lipo-oxidation (Bullock, 2003). In addition, a constituent of alligator pepper, beta-caryophyllene is a type 2 beta cannabinoid agonist, may activate type 2 beta cannabinoid, which accelerates lipid oxidation and results in fetal weight loss (Zheng *et al.*, 2013). Furthermore, Sugita *et al.* (2013) reported that orally ingested alligator pepper increases whole-body



energy expenditure through the activation of Brown Adipose Tissue in human subjects (Sugita *et al.*, 2013). This might also be another mechanism of action of the extract of alligator pepper for reducing litter weight, considering the fact that brown adipose tissue is preponderant in neonates. (Carter and Schucany, 2008)

## CONCLUSION

Intra-peritoneally administered aqueous extract of alligator pepper significantly reduces the litter weight of Alloxan induced diabetic rats and is therefore capable of preventing fetal macrosomia in pregnant Sprague Dawley rats with type 1 diabetes.

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Ehiagwina, E. carried out the research under the supervision of Inegbenebor, U. Both authors did the data analysis and article writing.

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