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Running Title: Hypoglycemic effects of *Aframomum melegueta* leaf extract on alloxan-induced diabetic rats

ABSTRACT

Aframomum melegueta (Zingiberaceae) seeds are used in West Africa, as a remedy for variety of ailments such as stomach ache, snakebite, diarrhea and anti-inflammatory properties. The hypoglycaemic effects of crude leaf extract of *Aframomum melegueta* on the treatment of alloxan induced diabetes in male rats and non-diabetic rats (control) were examined in this study. Results obtained from the experiment showed that the elevated blood glucose level caused by oral administration of 250 mg / kg body weight of alloxan was reduced significantly ($p < 0.01$) by oral administration of *Aframomum melegueta* leaf extract doses of 50, 100 and 200 mg/kg with the exception of 20 mg/kg when compared to control groups. The non-diabetic groups that received the extract showed reduction in blood sugar level as the dose increases when compared to their control group. There was a final weight gain and organ restoration for both the diabetic and non-diabetic rats after treatment when compared with their controls. This study showed that the extract have hypoglycemic and prophylactic effects.

Key words: *Aframomum melegueta* leaf, Alloxan, Diabetes, Hypoglycemia, male albino rats.

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INTRODUCTION:

Diabetes mellitus (DM) is a group of syndromes characterized by derangements in carbohydrate, fat and protein metabolism. DM may be defined as a syndrome characterized by hyperglycemia due to an absolute or relative lack of insulin and/or insulin resistance (depressed target cell sensitivity) [1, 2]. The general characteristics of DM include glucosuria, osmotic diuresis, polyuria, polydipsia and polyphagia.

DM may either be primary and secondary [3]. Primary DM is associated with inadequate insulin production, circulation or resistance to insulin action. Secondary DM maybe associated with existing disease conditions such as infections, pancreatic damage, stress or environmental conditions. Virtually all forms of DM are due to insulin deficiency and a decrease in the response of peripheral tissue of insulin (insulin resistance). These abnormalities lead to alteration in the metabolism of carbohydrates, lipids and ketones [4].

Control of diabetes by spices and other natural products is becoming popular and is more appropriate and economical for use in developing countries like Nigeria. Spices come from dried aromatic plants or trees and may be the bark, root, seeds, fruit, buds or the berry of these plants/ trees [2].

Aframomum melegueta is a West African plant, with common (local) names as Alligator pepper or Guinea pepper and Grain of paradise. It is a member of the family Zingiberaceae. It is about 1.0 m tall with narrow lanceolate bamboo like leaves at base of leafy shoots on very short peduncles, with bracts, and pink or lilac labellum. The bracts enclose the developing flowers. The fruit is ovoid with reddish colour and numerous small brownish angular seeds with a cardamom flavour. *Aframomum melegueta* are valued spice and this earned the plant the name 'Grains of paradise' [3]. They are also used for strengthening beer and other alcoholic drinks. In West Africa, the fruit pulp is chewed as a refreshing stimulant and the seeds and leaves are used for seasoning foods and in local medicine. It is also used as a remedy for variety of ailments such as snakebite, diarrhea, smallpox, chickenpox, wounds, cough, anaemia, rheumatism, measles, malaria, toothache, cardiovascular diseases, diabetics and fertility control [5, 6]. There have been claims by traditional herbalists that *Aframomum melegueta* can be used in treatment of diabetes.

The aim of this work was to investigate the hypoglycaemic effect of *Aframomum melegueta* (Alligator pepper) aqueous leaf extract on alloxan-induced diabetes mellitus in male albino rats.

MATERIALS AND METHODS:

Chemical

Alloxan was purchased from sigma chemical co U.S.A. All other chemicals used were of analytical grade.

Sourcing of Plant material

The leaves of *Aframomum melegueta* harvested in June were purchased from the local market in Lagos, Nigeria. It was authenticated by a taxonomist in the Botany Department of the University of Lagos. A voucher specimen was deposited (ascension number FHI108876) in the herbarium record.

Preparation of the aqueous extract of *Aframomum melegueta*

The leaves of *Aframomum melegueta* were sun dried and further dried in an oven at 25°C for two days to remove moisture content. The dried leaves were ground to powdery form with blender. A 100.0 g of the ground leaves were dissolved in 250 ml of distilled water. The mixture was filtered with muslin cloth. The filtrate was emptied into two beakers and evaporated to dryness. The concentrate was weight, and then dissolved in 50 ml of distilled water.

Induction of Experimental DM

The animals were fasted overnight and diabetes was induced by a modification of method described by Sekar et al. [7]. Briefly,

the animals were fasted overnight and DM was induced by a single oral administration of freshly prepared alloxan solution at a dose of 250 mg/kg body weight. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia [8]. After a week time for the development of DM, the rats with moderate DM having glycosuria and hyperglycemia (blood glucose range above 250 mg/dl) were considered diabetic and used for drug treatment. The aqueous leaf extract was then administered orally with various concentrations given daily according to the weight of each animal. The rats were weighed every three days during the duration period of the study.

Experimental animal /Study design

The 30 albino rats used for this study were obtained from the Laboratory animal's centre of the University of Lagos. They were acclimatized for two weeks, and fed rats pellets and water *ad libitum*. The rats were divided into 5 groups with 5 rats per group. Groups 1-4 were the diabetic induced rats that received oral doses of 20, 50, 100 and 200 mg/kg body weight of the aqueous extract respectively. The group 5 rats served as the control, received only distilled water. The aqueous extracts were given the first day, after which the blood glucose levels of the rats in the experimental and control groups were taken and recorded.

The administration of aqueous extracts continued after every 24 hours till the fifth day when the blood glucose measurements were repeated and the rats weighed. The rats were sacrificed on the sixth day, by cervical dislocation and blood samples obtained from ventricular punctures. The liver, pancreas and kidneys were harvested from each rat and weighed.

Estimation of blood glucose

Blood samples collected were used to estimate blood glucose levels using glucometer and strips. The Touch Basic made by Lifescan (Johnson & Johnson Company, California, USA) was used and the results were read off on the meter 45 seconds after application of blood samples to the strips [9]. The technical performance of the glucometer used was evaluated by comparison with standard laboratory method of blood glucose estimation (spectrophotometer) at the beginning, midway and at the end of the experiment as previously described by Ajala et al. [10].

Gravimetric analysis

The rats and the harvested organs were weighed at the end of experiment. Weighing was done using sensitive weighing machine [11] and values were expressed in grammes.

Statistical analysis

All results were analyzed using students t-test and ANOVA with the aid of SPSS (ver. 15) software package. The level of statistical significance was taken as $p < 0.05$. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals [12] and were approved by the Departmental Committee on the Use and Care of Animals in conformity with international acceptable standards.

RESULTS AND DISCUSSION:

The use of herbs is increasingly gaining acceptance among Africans and the world over as alternatives to orthodox medicine for the treatment of various types of diseases [13, 14, 15]. Regardless of the highly advanced orthodox medical therapies, substantial amount of medicinal plants are used for the treatment of ailments in some developed countries. In the United States of America, for example, medicinal plants constitute approximately 25% of all new refined prescriptions dispensed from community pharmacies [16]. The popularity of herbal product is being matched by a corresponding evidenced based research supporting their efficacy. *Aframomum melegueta* plant is one amongst such herbal products.

In the diabetic control group the increase in blood glucose level (Table 1) and reduction in weights of the pancreas, kidney and liver (Table 2) are indicative of the hyperglycemic effects of alloxan resulting from its ability to destroy the pancreatic islets cells. Our findings support recent report by Mahesar et al. [17] that administration of alloxan (150 mg/kg) led to about 3-fold elevation of fasting blood glucose levels, which was maintained over a period of 4 weeks.

Our data indicated that when treated with graded oral doses of the aqueous leaf extract (20, 50, 100 and 200 mg/kg), a dose related decrease in blood glucose level were observed for both the diabetic and non-diabetic rats compared to their control counterparts ($p < 0.05$). The *Aframomum melegueta* aqueous extract resulted in significant decrease ($p < 0.05$) in the blood glucose levels in the diabetic groups especially at highest dose of 200 mg/kg (Table 1). Although similar effects were also recorded in the non-diabetic groups significant reductions were observed more on alloxan-treated diabetic rats than non-diabetic rats (Table 3). Our results are similar to that obtained in a recent study by Ilic et al. [18] in which the effect of ethanol extract of the seeds of *Aframomum melegueta* caused reduction in blood glucose level in male diabetic rats. The lowest dose (20 mg/kg) of our aqueous leaf extract produced non-statistically significant decrease in blood glucose level ($p > 0.05$) in the diabetic rats. This may be due to the low

concentration of the active phytochemicals in the extract.

After the initial shrinking in weights observed in the pancreas, kidney and liver in the diabetic groups, the weights of these organs increased with administration of the aqueous leaf extracts (Table 2). These increases in weights might be due to the regeneration of organ tissues that were damaged by alloxan [19]. The aqueous leaf extract did not have significant effect on the body weights of the diabetic and non diabetic rats, however at a higher dose of 200 mg/kg there was significant increase in the weight of the diabetic rats (Tables 1). This pattern is similar to that reported by of Prohpl et al. [15] in which the weights of the diabetic and non diabetic animals did not change significantly at lower dose of the extracts after treatment.

The actual mechanism of action of the aqueous leaf extract is not fully understood. However, a possible mechanism may include direct inhibition of alloxan by competing with the glucose receptors on the β -cell membrane on the pancreas or by increasing the β -cell resistance by activation of super oxide dismutase which scavenges super oxide radicals [20]. This may also be a determining factor of the toxic effect of alloxan [20]. Further, the anti-hyperglycemic activity of the aqueous leaf extract maybe associated with an increase in plasma insulin level suggesting an insulinogenic activity; stimulating insulin secretion from

the remnant β -cells or from regenerated β -cells [8]. Though data from this study indicate that the aqueous leaf extract has significant hypoglycemic and prophylactic effects (suggesting it could be developed as a drug for treatment of diabetes), long term treatment

effect is yet to be ascertained. Further work should include estimation of the LD50, identification and isolation of the bioactive compound(s) and to elucidate their mechanism(s) of action.

Table 1: Effect of oral administration of *Aframomum melegueta* on blood glucose of diabetic and non diabetic rats¹ before and after treatment

Extract given in (mg/kg)	Non-diabetic group		Diabetic group	
	Initial mg/dl	Final mg/dl	Initial mg/dl	Final mg/dl
Control	39.00 ± 3.00	39.50 ± 3.50	90.50 ± 1.50	105.00 ± 5.00
20	53.66 ± 3.85	41.00 ± 4.89*	110.00 ± 5.71	110.30 ± 5.43
50	55.00 ± 10.42	39.00 ± 8.04*	104.00 ± 10.19	97.33 ± 9.97*
100	46.33 ± 5.79	39.00 ± 3.74	107.66 ± 8.37	76.66 ± 9.46*
200	56.00 ± 4.89	34.66 ± 5.24*	115.66 ± 3.68	48.00 ± 3.55**

¹Values represent Mean ± SD for rat and triplicate determination; * $p < 0.05$

Table 2: Effect of oral administration of *Aframomum melegueta* on the weight of some internal organs of diabetic and non diabetic rats¹

Concentration of extract (mg/kg)	Non-diabetic group (g)			Diabetic group (g)		
	Pancreas	Kidney	Liver	Pancreas	Kidney	Liver
Control	0.69±0.10	1.15±0.15	5.75± 0.04	0.24±0.04	0.55± 0.05	3.40± 1.05
20.00	0.71±0.10	1.17±0.12	5.76±1.02	0.44± 0.04	1.00± 0.21	6.03±1.72*
50.00	0.73±0.04	1.2± 2.10	6.00±0.80	0.51±0.09*	1.03±0.20*	5.26±0.57*
100.00	0.74±0.10	1.2± 0.10	7.36± 0.60	0.64±0.04*	1.20±0.08*	6.16±0.41*
200.00	0.8±0.10	1.36±0.12	7.8± 0.30	0.65±0.10*	1.33±0.09*	6.70±0.73*

¹Values represent Mean ± SD for rat and triplicate determination; * $p < 0.05$

Table 3: Effect of oral administration of *Aframomum melegueta* on body weight of diabetic and non diabetic rats¹

Doses of extracts (mg/kg)	Non-diabetic group		Diabetic group	
	Initial weight (g)	Final weight (g)	Initial weight (g)	Final weight (g)
Control	89.75 ± 2.15	89.35 ± 1.85	124.6 ± 5.60	112.25 ± 7.05
20.00	95.73 ± 14.21	88.00 ± 9.14	150.66 ± 11.89	148.76 ± 10.77
50.00	95.00 ± 6.09	87.03 ± 5.52	146.53 ± 24.27	143.93 ± 26.54
100.00	125.00 ± 26.99	133.40 ± 30.04	132.66 ± 35.33	137.76 ± 33.01
200.00	111.90 ± 15.94	114.60 ± 16.08	106.63 ± 37.93	123.4 ± 37.80*

¹Values represent Mean ± SD for rat and triplicate determination; **p* < 0.05 considered significant when compared to initial weight

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