Journal of Medicinal Plants Studies
www.PlantsJournal.com

**ISSN 2320-3862**
JMPS 2016; 4(5): 112-116

Received: 10-07-2016
Accepted: 11-08-2016

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# Responses of liver and pancreatic cells to ethanolic seed extract o*f Aframomum Melegueta* in alloxan-induced diabetic rats

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**Abstract**
Many traditional treatments have been recommended in the alternative system of medicine for the treatment of diabetes mellitus. *Aframomum melegueta*, a natural spicy and medicinal plant has been used traditionally in the treatment of diabetes mellitus. The purpose of this study was designed to evaluate scientifically the anti-diabetic activity of ethanolic seed extract of *A. melegueta (Alligator pepper)* in alloxan- induced diabetic rats. In doing so, the histopathology of the liver and pancreatic cells of the rats were studied. The animals were treated with the seed extract at doses of 200 and 400mg/kg and metformin (500mg/kg) the positive control orally daily for 7 days after induction of diabetes with Alloxan monohydrate (150mg/kg i.p). The results showed that the treated groups reduced blood glucose levels, reversed damaged liver cells and regenerated pancreatic β-cells than in the diabetic control group. Conclusively, the findings have shown that the seed extract may possess antioxidant, hypoglycemic and tissue protective properties thus supporting pharmacologically its folkloric use in the management of Diabetes Mellitus.

**Keywords:** *Aframomum melegueta*, alloxan, histopathology, liver, pancreas, rat

## 1. Introduction
Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population globally [1]. It is one of the leading causes of death in humans. It is a complex chronic disease that disturbs the metabolism of carbohydrate, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin [2, 3]. These alterations give rise to increased blood glucose, which cause long-term complications in many organs. Insulin is a hormone produced by the beta cells of the pancreas. These cells are sensitive to cytotoxic action of which induces experimental diabetes in animals [4]. The liver plays a major role in the metabolism of toxic substances (alloxan) that enter the body. The action of Alloxan in the pancreas is preceded by its rapid uptake by pancreatic beta cells suggesting that it may be one of the important features that leads to alloxan diabetogenicity. Alloxan is a hydrophilic and unstable chemical compound that has similar shape as that of glucose, which is responsible for this selective uptake and accumulation by the pancreatic beta cell. This shape allows it to be transported into the cytosol by the glucose transporter (GLUT2) in the plasma membrane of beta cell [5].

Several drugs such as biguanides (metformin) and sulfonylureas (glibenclamide) are presently available as antidiabetics and in spite of the progress in the management of diabetes using these synthetic drugs, many traditional plant treatments are still being used all over the world. Hence, plants are valued in traditional systems of medicine for the treatment of various diseases [6]. The ethnobotanical information reports state that about 800 plants may possess antidiabetic potential [7]. Some of the plants that are being used for the treatment of diabetes have received scientific and medicinal scrutiny. The World Health Organization expert committee on diabetes recommends that this area needs further attention [8]. Plants with hypoglycemic activity may act on blood glucose through different modes of action. Some may serve as antioxidants where they reduce oxidative stress that are due to free radicals in the pancreas [9-12], some may also inhibit insulinase activity or increase beta-cells in the pancreas by activating the regeneration of these cells [13] or some may contain some insulin-like substances [14] in reducing blood glucose.

*Aframomum melegueta* (alligator pepper) (Zingiberaceae) is a plant that possesses both medicinal and nutritive properties. The traditional names of the seed popularly known as alligator pepper in Nigeria are Ose-oji (Igbo), Atare (Yoruba), and Citta (Hausa). It is popularly used in herbal medicine against a wide range of ailments by many cultures in Africa especially in Nigeria. *Aframomum melegueta* is widely used as a spice in food, served and eaten during entertainment with kola nuts to visitors. Its aqueous extract has been shown pharmacologically to reduce gestational weight in pregnant rats [15]. The spice is used in West Africa for the purposes of alleviating stomachache and diarrhea, cardiovascular diseases, diabetes and inflammation [16], as an aphrodisiac [17] and a remedy for snakebites and scorpion stings [18]. Whole fruit eaten along with two moderately sized ginger cures beri beri. 1 whole pepper added to 3 seeds of ripe papaw, dried locus bean all ground to make soup is a remedy for female infertility [15]. The purpose of this study was to evaluate scientifically the anti-diabetic activity of *Aframomum melegueta* ethanolic seed extract and the histopathological effect on the liver and pancreatic cells in alloxan induced diabetic rats.

## Materials and Methods

### Animals

Adult wistar rats (120-200g) were procured from the Department of Physiology, University of Nigeria, Enugu Campus. Animals were housed in a well-ventilated cages at room temperature (25±5 $^0$C) under controlled lights cycles of 12 hrs light/dark. They were allowed access to feed and water *ad libitum*. All the rats were allowed a period of 14 days to acclimatize before the commencement of the experiment. Protocols of the experiments were approved by the Institutional Animal Ethics Committee.

### Sample collection, identification and preparation

The fruit were collected from Uturu village of Abia State identified as *Aframomum melegueta* at the Department of Plant Science and Biotechnology, Faculty of Biological and Physical Sciences, Abia state University Uturu, Nigeria. The dried fruits were cut open and the seeds were air dried for 7 days and pulverized into powder using a manual grinder to obtain a fine powder. This was packed in an air tight container and stored ready for extraction. The powdered seed was weighed and extracted each time with 98% (absolute) ethanol using Soxhlet extraction unit. The extract was evaporated using water-bath, dried in an oven, weighed (154g) and stored in an air tight container. For use each time, weighed crude extract was dissolved in 3ml of 3% Tween 80.

### Experimental protocols

Twenty five Wistar rats weighing 120 - 200g were categorized into five groups (A, B, C, D and E) rats. Each group contained 5 rats. Alloxan (150mg/kg b/w) was used to induce Diabetes in groups B, C, D and E by intraperitoneal injection after an overnight fast. Group A and B serving as Normal and Diabetic controls respectively, were given 0.2ml normal saline while groups C, D, and E received daily treatments of Metformin (500mg/kg b/w), 200 and 400mg/kg b/w of the extract respectively for 7 days. All the rats received food and water *ad libitum.* The weight (g) and glucose levels (mg/dl) of the rats were measured on day 0, 3 and 10 hence, glucose levels were estimated on these days using a One Touch Glucometer (Accu Check) by drawing blood from the tail of all the experimental animals. After expiration of the extract administration, rats were anaesthetized using Chloroform, while their peritoneal linings were stripped open to excise the liver and pancreas which were consequently prepared for histopathological studies using Hematoxylin and Eosin staining techniques. The effects of the extracts were compared with the reference drug, Metformin and the normal control.

## Results and Discussions

The result of the effect of *Aframomum melegueta* ethanol seed extract on glucose levels of experimental rats is shown in Table 1. The administration of alloxan elevated the blood glucose levels of the rats. Seven days of daily oral *A. melegueta* ethanol seed extract treatment (200 or 400mg/kg) or metformin (500mg/kg) significantly reduced the blood glucose levels of diabetic rats ($P \leq 0.05$). The blood glucose levels of Alloxan treated rats (diabetic control) increased about 3 folds from 94 - 296g/dl on the 10th day as compared with the control rats. Blood glucose levels in diabetic rats treated with the seed extract and metformin were raised nearly 3 folds as compared to their respective normal control rats on the 3rd day after induction of diabetes. The increase in glucose levels in diabetic rats declined sharply after daily oral administration of the ethanol seed extract of A. melegueta and Metformin. When glucose levels were compared between diabetic control and extract treated animals, blood glucose levels were found to decrease sharply from 243 - 138mg/dl on the 10th day after oral feeding of 200mg/kg, from 256 - 114mg/dl on the 10th day after oral feeding of 400mg/kg seed extract. Metformin treatment on the other hand decreased blood glucose levels on the 10th day from 297 - 104mg/dl.

**Table 1:** Effect of Ethanolic Seed Extract of Aframomum *Melegueta* on Glucose Level in Alloxan- Induced Diabetic Rats

| Treatment | Day 0 (mg/dl) | Day 3 (mg/dl) | Day 10 (mg/dl) |
|---|---|---|---|
| Alloxan only (Diabetic Control) | 94±8.48 | 297.83±5.42 | 296.67±6.72 |
| Alloxan + metformin (150mg/kg) | 94.33±6.41 | 297.67±8.68 | 104.00±14.80 |
| Alloxan + 200g of extract | 97.50±17.48 | 243.16±3.66 | 138.33±8.26 |
| Alloxan + 400g of extract | 107.67±13.00 | 256±43.26 | 114.33±7.53 |
| Normal saline (control) | 103.5±4.72 | 98.3±6.46 | 106.17±8.40 |

The response on pancreatic and liver cells showed various degree of abnormalities. The histology of the pancreas revealed that normal control group had normal and intact islet cells. (Plate1) However, alloxan-induced diabetic rats (diabetic control rats) showed necrosis (N) and atrophy of islet cells (N) (Plate II). Diabetic rats that had been treated with the 200mg/kg extract had or slight atrophy of islet cells (I A) which is an improvement from what occurred in the untreated alloxan-induced diabetic rats (Plate III). Group treated with 400mg/kg seed extract were observed to have improved the histology of the pancreas with normal intact islet cells as the concentration was increased (Plate IV). Metformin, the standard drug showed a recovery of the pancreatic tissues as there was normal intact islet cells (Plate V). Comparing the Liver histology of normal control rats (VI) and Diabetic control rats (Plate VII), kupffer cell hyperplasia (KC) with

sinusoidal congestion (SC) was evident in the untreated alloxanised rats (Diabetic control rats). However this was not the case with metformin treated rats (Plate VIII) where there was slight perivascular necrosis (PN) observed in the extract treated alloxanised rats, the 200mg/kg (Plate IX) showed intense pyknotic nuclei (PN) with kupffer cell hyperplasia (KC) and slight necrosis (N). This observation progressed dose and time dependently with slight improvement in 400mg/kg treated rats (Plate X) having kupffer cell hyperplasia (KC) with slight perivascular necrosis (PN).

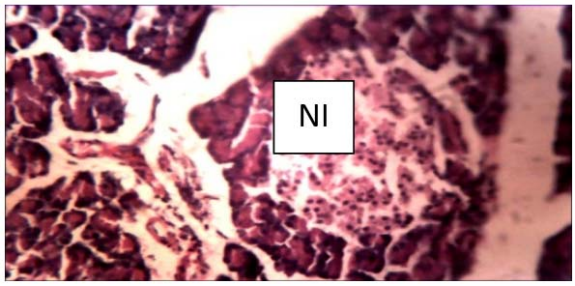


**Plate I:** Photomicrograph of a pancreatic section from the Normal control group showing normal islet (NI), H/E staining, 250x

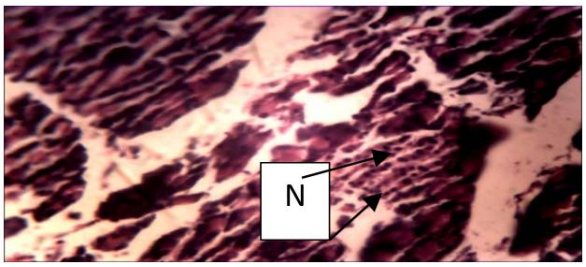


**Plate II:** Photomicrograph of a pancreatic section from the Alloxan treated (diabetic control) group showing necrosis and atrophy of islet (N). H/E staining, 250x

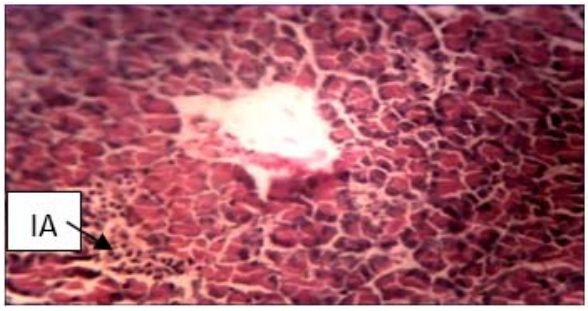


**Plate III:** Photomicrograph of a pancreatic section from the Alloxan treated (200mg/kg *A. melegueta* seed extract) group showing islet atrophy (IA). H/E staining, 250x

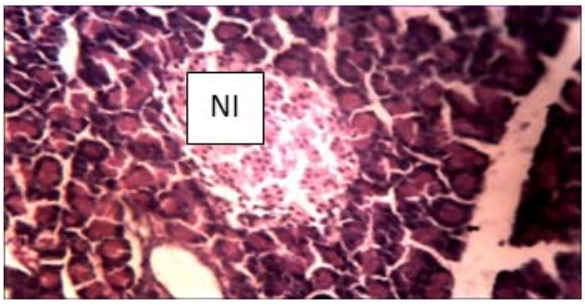


**Plate VI:** Plate V. Photomicrograph of a pancreatic section from the Alloxan treated (400mg/kg *A. melegueta* seed extract) group showing normal islet (NI), H/E staining, 250x

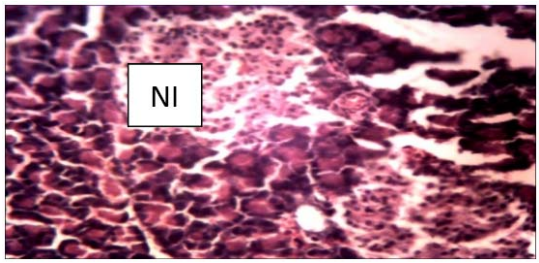


**Plate V:** Photomicrograph of a pancreatic section from the Alloxan treated (Metformin 500mg/kg) group showing normal islet (NI), H/E staining, 250x

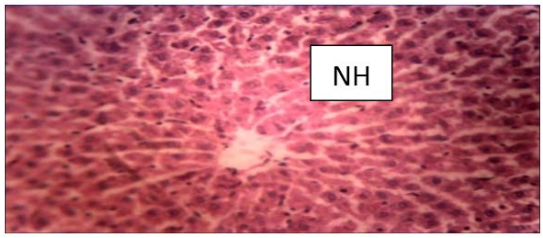


**Plate VI:** Photomicrograph of a liver section from the Normal control group showing normal hepatocyte (NH), H/E staining, 250x

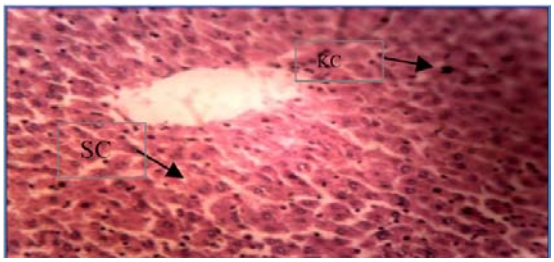


**Plate VII:** Photomicrograph of a liver section from the Alloxan treated (diabetic control) group showing kupffer cell hyperplasia (KC), sinusoidal congestion (SC); Hematoxylin and Eosin staining. 250x

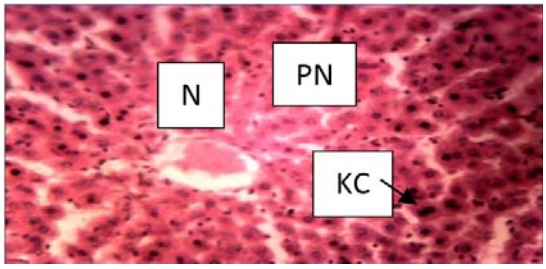


**Plate VIII:** Photomicrograph of a liver section from the Alloxan treated (200mg/kg *A. melegueta* seed extract) group showing pyknotic nuclei (PN), kupffer cell hyperplasia (KC) and necrosis (N). H/E staining, 250x

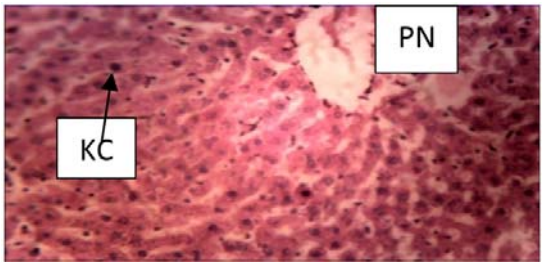


**Plate IX:** Photomicrograph of a liver section from the Alloxan treated (400mg/kg *A. melegueta* seed extract) group showing slight perivascular necrosis (PN), kupffer cell hyperplasia (KC), H/E staining, 250x

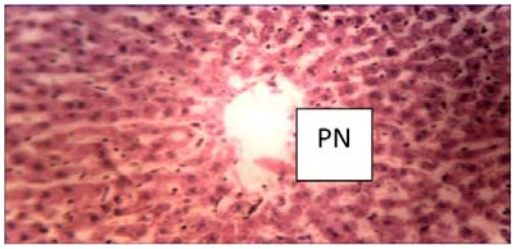


**Plate X:** Photomicrograph of a liver section from the Alloxan treated (Metformin 500mg/kg) group showing perivascular necrosis (PN), H/E staining, 250x

This study showed a dose dependent decrease in blood glucose levels of ethanol seed extract of *A. melegueta*. This result may indicate that *A. melegueta* possess antihyperglycemic ingredients which has been shown by other workers that used different solvent extractions. The tissue protective effects of some natural compounds isolated from roots, bark, stem, fruit and seeds were believed to be associated with their antioxidant activity [11, 10]. The result of the phytochemical screening of the seeds of *Aframomum melegueta* revealed the presence of alkaloids, flavonoids, tannins, saponin, steroids, cardiac glycosides and terpenes [19]. It has been reported that flavonoids, tannins, and saponins possess hypoglycaemic properties through an inhibitory action on the sodium-glucose transporter 1 (S-GLUT1) [20]. Alloxan, a cytotoxic agent may have damaged the tissues via oxidative stress. There is a growing scientific evidences that excess generation of highly reactive free radicals, largely due to hyperglycemia, cause oxidative stress, which further increases the development and progression of diabetic complications [21] in this case, the tissue damage. Flavonoids which are powerful antioxidant may be responsible for the tissue protective effect on the organs damaged by alloxan [22]. It has also been reported that saponins possess hypoglycaemic activity, which may be due to the inhibition of liver glycogenesis or glycolysis [23]. This may have been the reason for the exhibition of the hypoglycaemic activity by the plant extract.

Histopathological studies of diabetic control rats showed necrosis and atrophy of the Islet cells of the pancreas leading to the degeneration of the cells. This could be because the action of Alloxan on the pancreas is preceded by its rapid uptake by pancreatic beta cells that has been proposed to be one of the important features for its diabetogenic actions. Since alloxan is a hydrophilic and unstable chemical compound that has similar shape as that of glucose, it may have resulted in its selective uptake and accumulation by the pancreatic beta cell. The shape also allows it to be transported into the cytosol by the glucose transporter (GLUT2) in the plasma membrane of beta cell [5]. The responses of the treated groups (200 and 400mg/kg and Metformin 500mg/kg) showed an increased dense volume of Islet cell which is a sign of regeneration. The occurrence of regeneration of β-cells and a decrease in blood glucose levels have been reported when some plant extracts have been consumed [4]. This showed that the seed of *A. melegueta* may have some chemical ingredients that produce regenerative effects on β-cells, stimulate these cells to produce more insulin or have some insulin-like substances because higher dose (400mg/dl) of the extract showed a greater restorative effect on the islet cells of diabetic control rats when compared with extract of lower dose. The histology of the liver of diabetic control rats also showed necrosis which could be due to generation of oxygen free radical. Alloxan causes liver and pancreatic beta cell toxicity and diabetogenicity that may be attributed to alloxan–induced redox cycling and ROS generation[5]. The tissue-protective effect of *A. melegueta* which was dose dependent can be observed by its ability in restoring and regenerating the already damaged tissues of alloxan induced rats. This could be due to its antioxidant effect on the tissues which prevented damage by oxygen-free radicals.

## Conclusion

The extract from seeds of *Aframomum melegueta* was observed to possess anti-diabetic and tissue-protective effects on rats, thus this supports pharmacologically the suggested folkloric use of this plant in the management of Diabetes Mellitus.

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