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Ameliorating effect of nutmeg *(Myristica fragrans)* on the pancreas of alloxan-induced diabetic rats

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Abstract

Objective: This research work was carried out to investigate the effect of Nutmeg on the histology of pancreas of alloxan-induced diabetic rats.

Methodology: Twenty-five Wistar rats weighing 150 g – 180 g were purchased and acclimatized for two weeks. Thereafter, they were divided into five groups designated as A – E with five rats each. Group A serve as the control group, while groups B – C served as the experimental group, and were induced with alloxan. Group A received distilled water only, while groups B – C received Glucophage, 400 mg/kg of Nutmeg and 800 mg/kg of Nutmeg daily for 21 days through orogastric tube. On the 22nd day, the animals were sacrificed via chloroform inhalation, and pancreas were harvested from the rats for histological study.

Results: Histopathological findings revealed normal well perfused pancreatic tissue with active Islets of Langerhans (IL), pancreatic acini (PA) and well out-lined acini cells (AC); poorly perfused pancreatic tissue with severe focal areas of Islets of the Langerhans atrophy and severe dissolution of acini; moderate necrotic (N) and pale appearance of the pancreatic acini in R1 however the Islets of the Langerhans in both sections are moderately normal; mild necrotic (N) and pale appearance of the pancreatic acini with mild aggregation of inflammatory cells (AIC); and mild aggregation of inflammatory cells (AIC) with active Islets of the Langerhans (IL) respectively for groups A – E.

Conclusion: Nutmeg extract has ameliorating effect on the histology of the pancreas of alloxan-induced rats which improves with increase in the dosages of the extract.

Keywords: Nutmeg; Pancreas; Diabetes; Alloxan.

1. Introduction

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin (a hormone that regulates blood glucose) it produces, [1] which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves [2]. It is characterized by high blood glucose levels with increased thirst, increased urination and increased hunger [3]. Its other symptoms include blurred vision, drowsiness, nausea and decreased endurance during exercise [3]. According to Erika [3], when the blood glucose level rises above 160 to 180 mg/dL (8.9 to 10.0 mmol/L), glucose spills into the urine, and also, when the level of glucose in the urine rises even higher, the kidneys excrete additional water to dilute the large amount of glucose. Because the

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kidneys produce excessive urine, people with diabetes urinate large volumes frequently (polyuria), thus creating abnormal thirst (polydipsia); and because excessive calories are lost in the urine, people may lose weight, and often feel excessively hungry to compensate for the lost calories [3]. Subclassifications of diabetes mellitus include type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and steroid-induced diabetes [4]. Type 1 and 2 DM are the main subtypes, each with different pathophysiology, presentation, and management, but both have a potential for hyperglycemia [4].

Diabetes is the direct cause of 1.5 million deaths in 2019, and 48% of all deaths due to diabetes occurred before the age of 70 years; and also, another 460 000 kidney disease deaths were caused by diabetes, while raised blood glucose causes around 20% of cardiovascular deaths [5]. It is a dangerous condition because it can exist undetected for years, during this time, many conditions can develop as a result of this underlying problem, and is also associated with long-term complications that affect almost every part of the body, such as blindness, heart and blood vessel disease, stroke, kidney failure, amputations and nerve damage [6]. Its major long-term complications is damage to blood vessels, thus doubling the risk of cardiovascular disease, and about 75% of deaths in people with diabetes are due to coronary artery disease [7]. Other macrovascular diseases include stroke and peripheral artery disease [8]. The primary complications of diabetes are due to damage in small blood vessels including damage to the eyes, kidneys, and nerves [9]. Research has shown that about 422 million people worldwide have diabetes, the majority living in low-and middle-income countries, and 1.5 million of deaths are directly attributed to diabetes each year [2]. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades [2].

The pancreas is an organ of the digestive and endocrine system of vertebrates [10]. It sits behind the stomach, with the body near the curvature of the duodenum, and its tail stretching to touch the spleen. It is an organ in humans that lies in the abdomen, stretching from behind the stomach to the left upper abdomen near the spleen. In adults, it is about 12–15 cm (4.7–5.9 in) long, lobulated, and salmon-colored in appearance [11]. Anatomically, the pancreas is divided into a head, neck, body, and tail. It stretches from the inner curvature of the duodenum, where the head surrounds two blood vessels: the superior mesenteric artery and vein. The longest part of the pancreas, the body, stretches across behind the stomach, and the tail of the pancreas ends adjacent to the spleen [11]. It is involved in blood sugar control and metabolism within the body, and also in the secretion of substances (collectively known as the pancreatic juice) that help digestion. These roles are divided into an "endocrine" role, relating to the secretion of insulin and other substances within pancreatic islets that help control blood sugar levels and metabolism within the body, and an "exocrine" role, relating to the secretion of enzymes involved in digesting substances in the digestive tract [12].

The pancreas contains tissue with an endocrine and exocrine role, and this division is also visible when the pancreas is viewed under a microscope [12]. The majority of pancreatic tissue has a digestive role. The cells with this role form clusters (Latin: acini) around small ducts, and are arranged in lobes that have thin fibrous walls. The cells of each acinus secrete inactive digestive enzymes called zymogens into the small intercalated ducts which they surround. In each acinus, the cells are pyramidal in shaped, and situated around the intercalated ducts, with the nuclei resting on the basement membrane, a large endoplasmic reticulum, and a number of zymogen granules visible within the cytoplasm. The intercalated ducts drain into larger intralobular ducts within the lobule, and finally interlobular ducts. The ducts are lined by a single layer of column-shaped cells. There is more than one layer of cells as the diameter of the ducts increases [12].

The tissues with an endocrine role within the pancreas exist as clusters of cells called pancreatic islets (also called islets of Langerhans) that are distributed throughout the pancreas [12]. Pancreatic islets contain alpha cells, beta cells, and delta cells, each of which releases a different hormone. These cells have characteristic positions, with alpha cells (secreting glucagon) tending to be situated around the periphery of the islet, and beta cells (secreting insulin) more numerous and found throughout the islet [12]. Enterochromaffin cells are also scattered throughout the islets [12]. Islets are composed of up to 3,000 secretory cells, and contain several small arterioles to receive blood, and venules that allow the hormones secreted by the cells to enter the systemic circulation [12]. A chronic autoimmune disease in which the immune system attacks this insulin-secreting beta cells of the pancreas results to diabetes mellitus type 1 [13]. Also diabetes mellitus type 2 which is the most common form of diabetes [12] may result from a combination of insulin resistance and impaired insulin secretion, with both genetic and environmental factors destroying the beta cells of the pancreas [14]. Over time, pancreatic beta cells may become "exhausted" and less functional [12].

Alloxan which is also known as alloxan hydrate, is an organic compound with the formula $OC(N(H)CO)_2C(OH)_2$ [15]. It was originally obtained by oxidation of uric acid by nitric acid, and is prepared by oxidation of barbituric acid by chromium trioxide [16]. It is a toxic glucose analogue, which selectively destroys beta cells in the pancreas when administered to rodents and many other animal species [15]. This causes an insulin-dependent diabetes mellitus called "alloxan diabetes" in these animals, with characteristics similar to type 1 diabetes in humans [12]. It is selectively toxic

to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter [15]. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction [12].

Because alloxan selectively kills the insulin-producing beta-cells found in the pancreas, alloxan is used to induce diabetes in laboratory animals [17, 18]. This occurs most likely because of selective uptake of the compound due to its structural similarity to glucose as well as the beta-cell's highly efficient uptake mechanism (GLUT2) [15]. In addition, alloxan has a high affinity to SH-containing cellular compounds and, as a result, reduces glutathione content [15]. Furthermore, alloxan inhibits glucokinase, an SH-containing protein essential for insulin secretion induced by glucose [19]. Most studies have shown that alloxan is not toxic to the human beta-cell, even in very high doses, probably because of differing glucose uptake mechanisms in humans and rodents [20, 21]. Alloxan is, however, toxic to the liver and the kidneys in high doses [15]. Research has shown that alloxan-induced destruction of the pancreatic islet beta cell produces permanent diabetes mellitus in a wide range of species and, in the appropriate dose, it is selective to islet beta cell, producing minimal effects on other structures [22]. Also, alloxan-induced diabetes triggered liver morphological and ultrastructural changes that closely resembled human disease, ranging from steatosis to steatohepatitis and liver fibrosis [23].

Nutmeg (*Myristica fragrans*) is a tropical evergreen tree that belongs to the family *Myristicaceae* [24]. It is a dark-leaved evergreen tree cultivated for two spices derived from its fruit: nutmeg, from its seed, and mace, from the seed covering; and is also a commercial source of nutmeg essential oil and nutmeg butter. Indonesia is the main producer of nutmeg and mace, and the true nutmeg tree is native to its islands. Conifers of the genus *Torreya*, commonly known as the nutmeg yews, have edible seeds of similar appearance, but are not closely related to *M. fragrans*, and are not used as a spice. Nutmeg is the spice made by grinding the seed of the fragrant nutmeg tree (*Myristica fragrans*) into powder. The spice has a distinctive pungent fragrance and a warm, slightly sweet taste; it is used to flavor many kinds of baked goods, confections, puddings, potatoes, meats, sausages, sauces, vegetables, and such beverages as eggnog [25]. The seeds are dried gradually in the sun over a period of six to eight weeks. During this time, the nutmeg shrinks away from its hard seed coat until the kernels rattle in their shells when shaken. The shell is then broken with a wooden club and the nutmegs are picked out. Dried nutmegs are grayish brown ovoids with furrowed surfaces [25]. The nutmegs are roughly egg-shaped, about 20.5–30 mm (0.81–1.18 in) long and 15–18 mm (0.59–0.71 in) wide, weighing 5–10 g (0.18–0.35 oz) dried.

It is a rich source of antioxidants that help to protect against the signs of aging and serious conditions such as cancer, heart disease, and liver disease [26]. Its oil has been shown to be used in several dental products, and the spice has anti-bacterial properties that have proven particularly effective against oral pathogens that cause disease and bad breath [26]. Also, a little nutmeg has been shown to aid sleep, both in duration and quality [26]. *M. fragrans* is rich in fiber, which helps to keep the digestive system healthy, and prevent blood sugar from spiking, and is also a source of Vitamin A, Vitamin C, Vitamin E, Manganese, Magnesium, Copper, Phosphorous, Zinc and Iron [26]. According to Gupta *et al.,* [27] it contains the following antioxidants - vitamins, carotenoids (beta-carotene and beta-cryptoxanthin), terpenoids, alkaloids, flavonoids, lignans and phenolic compounds. It also contains micronutrients calcium and magnesium, which are important in regulating blood pressure [28]. Traditionally, it has been used in Ayurvedic (traditional Indian), traditional Chinese, and traditional Thai medicines due to its strong antioxidant and antimicrobial properties [29].

The seed of nutmeg is rich in essential oils [30]. It is a bitter, astringent, spicy herb that acts as a warming, digestive tonic [30]. It controls vomiting and relaxes spasms [30]. When applied externally, it has an anti-inflammatory effect [30]. Nutmeg is also said to have stimulant, carminative and aphrodisiac properties [31]. Externally, the seed is used to treat toothache, rheumatic and abdominal pains (including labour pains) [30]. Some caution is advised - taken in excess the seed can cause severe headache, nausea, dizziness and delirium [30]. It is used in Ayurveda to treat poor digestion, insomnia, urinary incontinence and premature ejaculation [30]. Nutmeg can be used as a narcotic with hallucinogenic effects but it is dangerous; the consumption of two ground nutmegs (about 8 g) is said to cause death, due to its myristicin content. On Zanzibar nutmegs are chewed as an alternative to smoking marihuana [31].

When ingested in small amounts as a spice, it produces no noticeable physiological or neurological response, but in large doses, both raw nutmeg freshly ground from kernels and nutmeg oil have psychoactive effects [32. 33]. which appear to be derived from anticholinergic-like hallucinogenic mechanisms attributed to myristicin and elemicin [34. 35]. *Myristicina* monoamine oxidase inhibitor and psychoactive substance [32., 34] can cause convulsions, palpitations, nausea, eventual dehydration, and generalized body pain when consumed in large amounts [32. 33]. Its usage may increase endocannabinoids like anandamide and 2-AG levels or delay their breakdown by inhibiting FAAH and MAGL [36]. It may

interact with anxiolytic drugs, produce allergic reactions, cause contact dermatitis, and evoke acute episodes of psychosis [32].

Varying considerably from person to person, nutmeg intoxication may occur with side effects, such as delirium, anxiety, confusion, headaches, nausea, dizziness, dry mouth, eye irritation, and amnesia [32. 34]. Nutmeg intoxication takes several hours to reach maximum effect [32] and its effects may last for several days [34, 33]. Rarely, nutmeg overdose causes death, especially if the nutmeg is combined with other drugs [34]. Incidents of fatal poisoning from nutmeg and myristicin individually are uncommon [32]. Its poisonings occur by accidental consumption in children and by intentional recreational use [34]. It is used recreationally with the intention of achieving a low-cost high resembling psychedelic, particularly by adolescents, drug users, college students, and prisoners [37]. Relatively large doses of nutmeg are required to produce effects; a majority of reported nutmeg intoxication cases appear to be the result of recreational use [38].

Nutmeg was once considered an abortifacient, but may be safe during pregnancy if used only in flavoring amounts [32]. If consumed in large amounts, nutmeg could cause premature labor and miscarriage. Nutmeg may also interact with pain relievers such as pethidine, so avoiding it during pregnancy is recommended [39]. The scent of nutmeg may attract pets, but it can be poisonous to them if they consume too much [40].

Thus, this study will help to create awareness on the ameliorating effect of Nutmeg on the histology of the pancreas of alloxan-induced diabetic rats.

2. Material and methods

2.1. Animal procurement, care, and treatment

Twenty (25) male rats which weighed between 160 g - 180 g were procured from Nnamdi Azikiwe University, Nnewi Campus, and housed at the Animal house of Anatomy Department, Abia State University, Uturu with wire gauze cages in a well-ventilated area. They were maintained under standard laboratory conditions of temperature ($22+2^0$), relative humidity (55-65%), 12 hours of light/dark cycle [41], and were fed with standard commercial pellet diet and water *ad libitum*. There were acclimatized for two weeks before the experiment and their health statuses were closely monitored before and during the experiment. All procedures were carried out in strict accordance with the Institutional guidelines on the care and use of experimental animals.

2.2. Collection, identification, and preparation of seed material

Nutmegs were purchased from a local Market known Eke Okigwe Market in Okigwe Local Government Area of Imo State because of its proximity to the researchers. The seeds were authenticated at Herbarium unit, Botany Department, Abia State University, Uturu, Abia State. The seeds were powdered and subjected to extraction with petroleum ether (60 – 80 °C) using soxhlet apparatus [42]. The extract was then concentrated to dryness under reduced pressure in rotary vacuum evaporator and later used in the experiment. The percentage yield of prepared extract was around 16% w/w [42].

2.3. Induction of alloxan

The rats were divided into non-diabetic control group and experimental groups. The baseline blood glucose level of the experimental group to be inducted was determined before the induction of diabetes. The rats were allowed to fast over-night prior to injection of alloxan and diabetes was induced by intra-peritoneal administration of 150 mg of alloxan per kg body weight of rat (150 mg/kg body weight) [43]. After the induction, the rats were allowed to have free access to the same feed and water. After 72 hours, blood samples obtained through tail tip puncture of the rats were used to confirm diabetes in the rats by testing for hyperglycemia using Glucometer. Diabetes was confirmed at fasting blood glucose levels greater than 200 mg/dl [44].

2.4. Experimental protocol

The animals were grouped into five (5) groups of five (5) rats each. Different doses of the Nutmeg oil were administered via oral route with the aid of oral gastric tube as shown below:

- Group A (The Control group) distilled water.
- Group B (Diabetic group) distilled water.
- Group C Diabetic + Glucophage

- Group D Diabetic + 400 mg/kg seed extract of Nutmeg.
- Group E Diabetic + 800 mg/kg seed extract of Nutmeg.

2.5. Sample collection and analysis

The extracts were administered for twenty-one (21) days. On the 22nd day, the animals were sacrificed by anaesthetizing under chloroform vapour and dissected. Pancreases were harvested from the animals, and were fixed in 10 % formal saline for four hours. This was followed by histological and histochemical methods of tissue processing.

3. Results

3.1. Histopathological findings

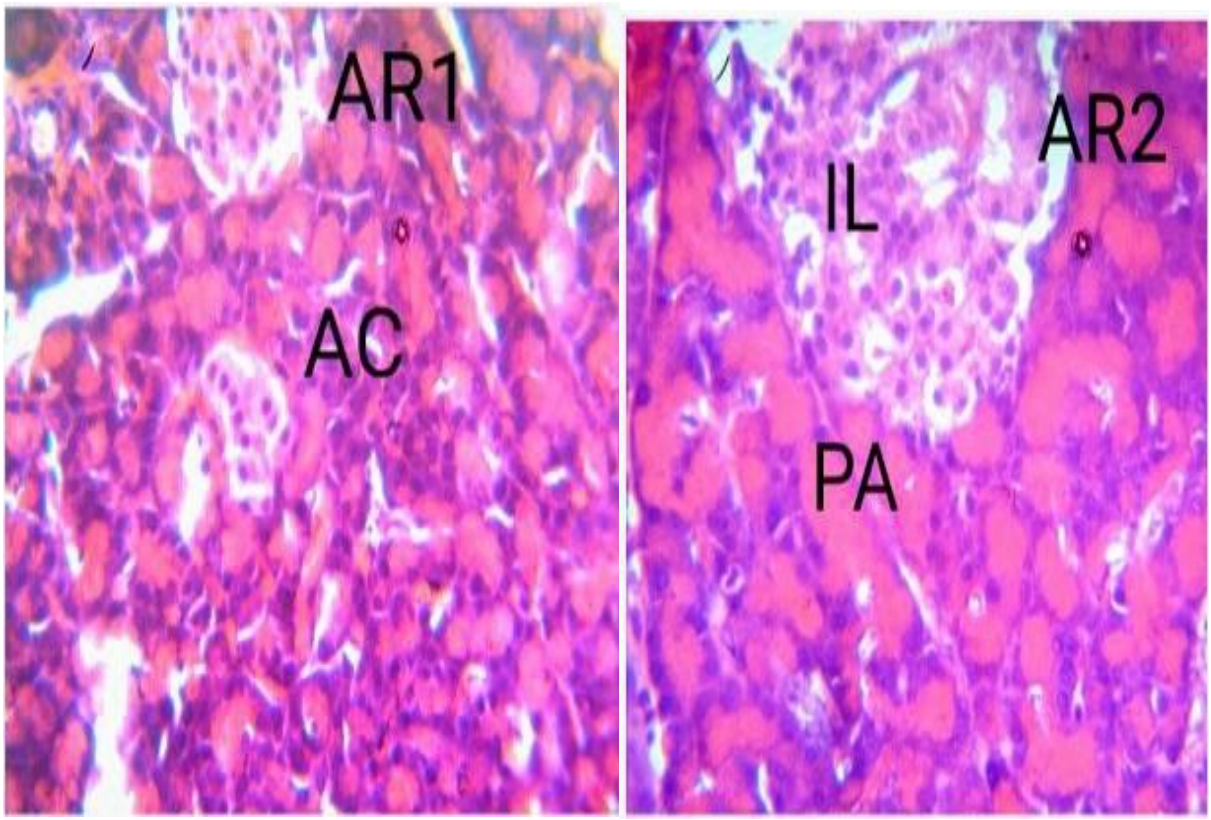

Figure 1 This is a photomicrograph of group A (AR1R2) control section of pancreas (x400) (H/E) showing normal well perfused pancreatic tissue with active Islets of Langerhans (IL) pancreatic acini (PA) and well outlined acini cell (AC).

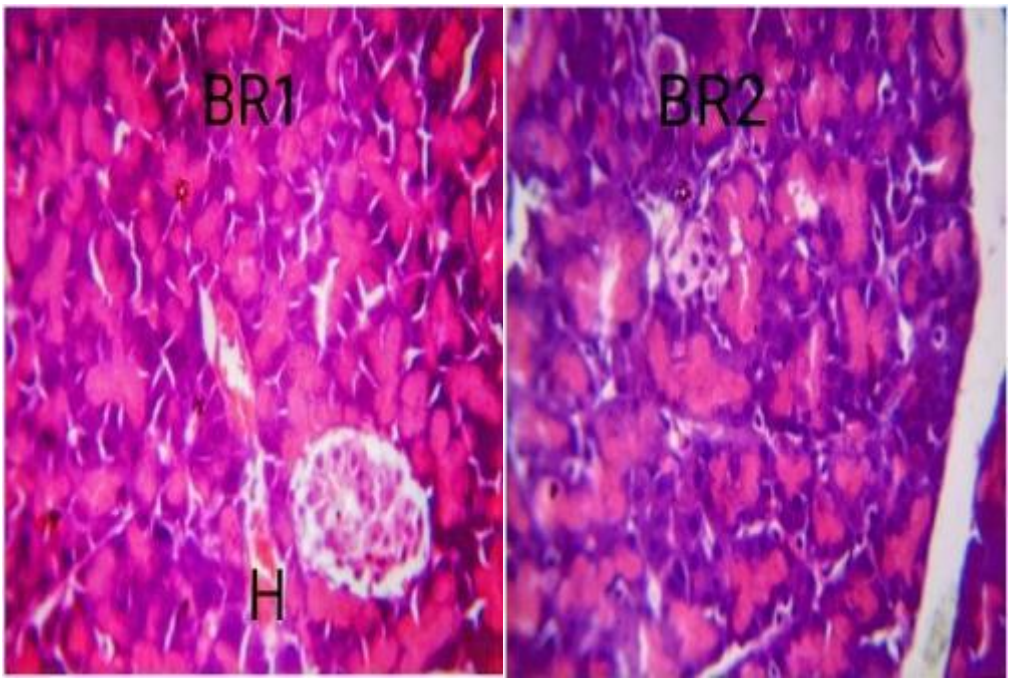

Figure 2 This is a photomicrograph of group B (BR1R2) section of pancreas induced and treated with Glucophage (x400) (H/E) showing moderate regeneration with mild focal area of hemorrhage (H) otherwise normal.

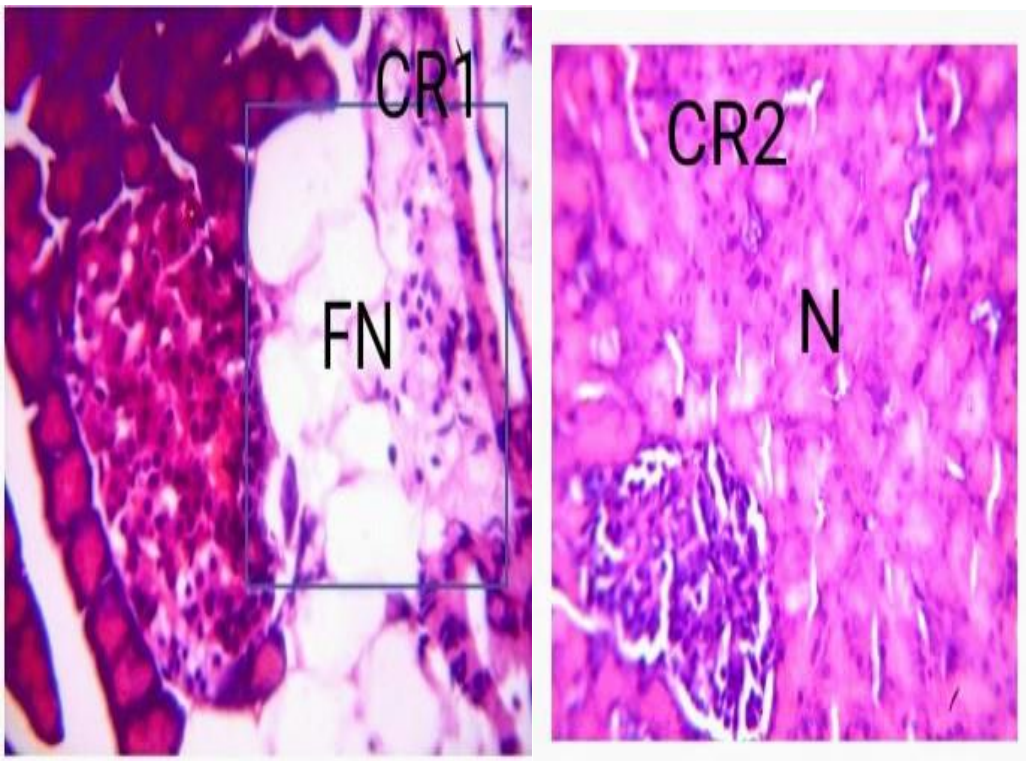

Figure 3 This a photomicrograph of group C (CR1R2) section of pancreas induced alloxan only (x400) (H/E) showing severe degeneration with focal area of fatty necrosis (FN) and severe necrotic (N) pancreatic acini with non-acini cell outline.

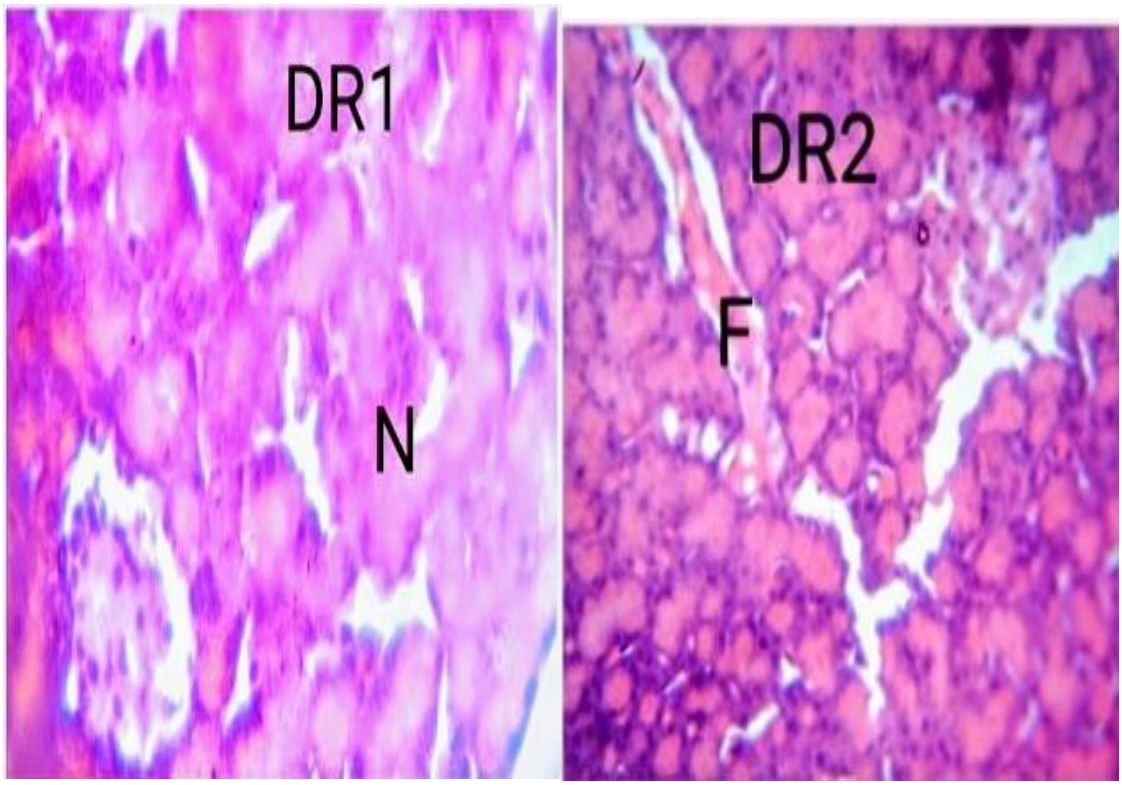

Figure 4 This is a photomicrograph of group D (DR1R2) section of pancreas induced with alloxan and treated with 400 mg/kg of seed extract of Nutmeg (x400) (H/E) showing moderate regeneration with mild necrotic (N) pancreatic acini in R1 with non-distinct pancreatic cell outline and fibrosis (F) in R2.

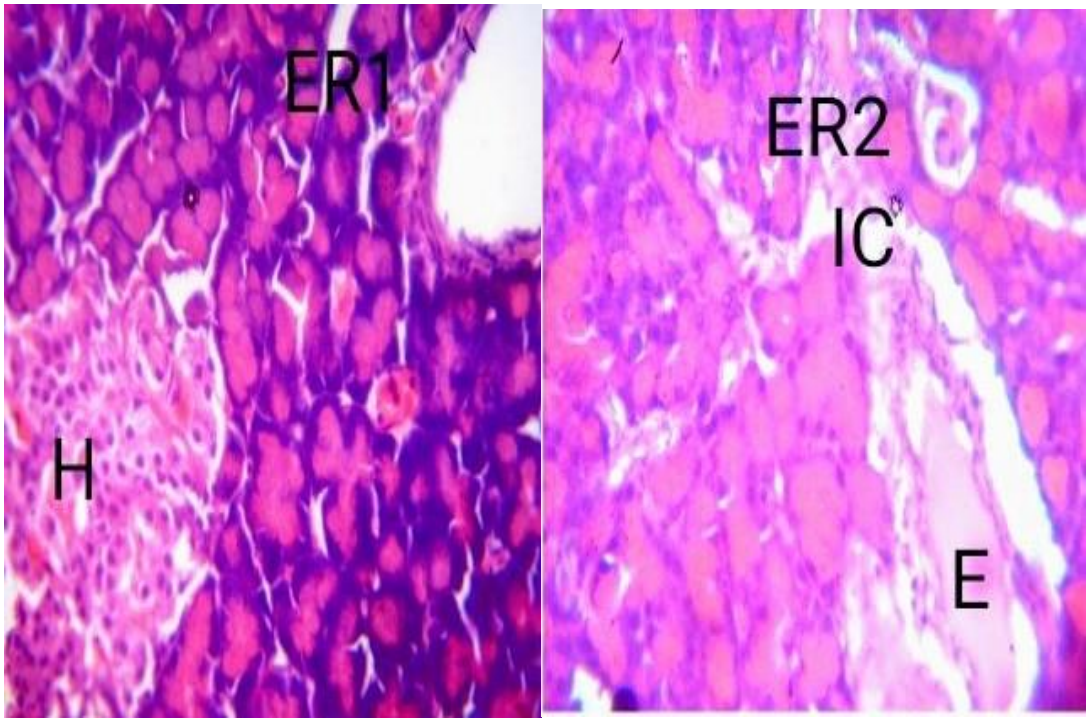

Figure 5 This is a photomicrograph of group E (ER1R2) section of pancreas induced with alloxan and treated with 800 mg/kg of seed extract of Nutmeg (x400) (H/E) showing mild regeneration with mild hemorrhagic Islet (H) in R1 and moderate focal area of edema (E) surrounded by inflammatory cell (IC) in R2.

4. Discussion:

In figure 1 group A (AR1R2), the control section of pancreas (x400) (H/E) showed normal histoarchitecture of well perfused pancreatic tissue with active Islets of Langerhans (IL) pancreatic acini (PA) and well outlined acini cell (AC). According to Barbara *et al.*, [12] pancreatic islets contain alpha cells, beta cells, and delta cells, each of which releases a different hormone. These cells have characteristic positions, with alpha cells tending to be situated around the periphery of the islet, and beta cells (secreting insulin) more numerous and found throughout the islet.

The photomicrograph of group B (BR1R2) section of pancreas induced and treated with Glucophage (x400) (H/E) of figure 2 showed moderate regeneration with mild focal area of hemorrhage (H) otherwise normal. This could be due to the Glucophage administered to the rats. Research has shown that Glucophage contains Metformin which is a type of medicine known as a biguanide, and it works to lower the amount of sugar in the blood of people with diabetes by lowering the amount of sugar produced in the liver, and also increasing the sensitivity of muscle cells to insulin [45].

In Figure 3; the photomicrograph of group C (CR1R2) section of pancreas induced alloxan only (x400) (H/E) showed severe degeneration with focal area of fatty necrosis (FN) and severe necrotic (N) pancreatic acini with non-acini cell outline. The pathological effect seen could be due to the induced alloxan. Research has shown that alloxan selectively destroys beta cells in the pancreas when administered to rodents and many other animal species [15] causing an insulin-dependent diabetes mellitus called "alloxan diabetes" in these animals, with characteristics similar to type 1 diabetes in humans [15]. This occurs most likely because of selective uptake of the compound due to its structural similarity to glucose as well as the beta-cell's highly efficient uptake mechanism (GLUT2) [15].

The photomicrograph of group D (DR1R2) section of pancreas induced with alloxan and treated with 400 mg/kg of seed extract of Nutmeg (x400) (H/E) showed moderate regeneration with mild necrotic (N) pancreatic acini in R1 with non-distinct pancreatic cell outline and fibrosis (F) in R2 of figure 4; while that of the photomicrograph of group E (ER1R2) section of pancreas induced with alloxan and treated with 800 mg/kg of seed extract of Nutmeg (x400) (H/E) showed mild regeneration with mild hemorrhagic Islet (H) in R1 and moderate focal area of edema (E) surrounded by inflammatory cell (IC) in R2 of figure 5. This could be due to the fact that nutmeg extract has potent antidiabetic and β-cell protection activities in alloxan induced diabetic rats, possibly via its antioxidant properties [42, 46]. The effects are dose dependent.

5. Conclusion

Nutmeg extract has ameliorating effect on the histology of the pancreas of alloxan-induced rats which improves with increase in the dosages of the extract.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There was no conflict of interest.

Statement of ethical approval

This research work was approved by the Ethical Approval Committee, Basic Medical Sciences, Abia State University, Uturu, Abia State, Nigeria.

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