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Effects of Combined Aqueous Seed Extract of *Monodora myristica* and *Xylopia aethiopica* on the Histology of Pancreas of Wistar Rats

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ABSTRACT

Monodora myristica and *Xylopia aethiopica* have been commonly used as spices for decades, both in culinary and medicinal contexts. This study aimed to assess the effect of aqueous extract of *Monodora myristica* and *Xylopia aethiopica* on the histology of the pancreas of adult albino Wistar rats. Twenty (20) rats weighing 160-180 g were divided into four groups of five rats each and labelled Group A, B, C, and D. Group A rats which served as control were solely provided with feed and water, while Group B received 500 mg/kg of the extract of *Monodora myristica*. Group C received 500 mg/kg of the extract of *Xylopia aethiopica*, and Group D received a combined extract of 500 mg/kg of *Monodora myristica* and 500 mg/kg of *Xylopia aethiopica* extracts. The rats received the extract for eight weeks daily and were sacrificed 24 hours after the last administration and routinely processed histologically. The histological assessment showed normal pancreatic features in group A. Group B showed congested blood vessels and hypertrophy of acinar cells. Group C exhibited alterations in the morphology of the islet cells and cytoplasm. Lastly, group D showed signs of periductal fibrosis in the interlobular duct with congested blood vessels. Therefore, the result suggests that *Monodora myristica* and *Xylopia aethiopica* may have an adverse effect on the pancreas cytoarchitecture, particularly the islet of Langerhans.

Keywords: *Monodora myristica*, *Xylopia aethiopica*, pancreas, cytoarchitecture

INTRODUCTION

Phytomedicine has been employed in health practices as its knowledge has been handed down through the decades and has incorporated plant, animal, and mineral-based medicines, spiritual therapies, manual techniques, and exercise, applied singularly or in combination to treat, diagnose, and prevent illnesses or maintain well-being[1]. In the world today, studies have been carried out globally to verify the efficacy, and some of the findings have led to the discovery and production of plant-based medicines, recipes, or remedies[2]. Some plants that exhibit medicinal properties have been known to help stabilize different internal organs in animals and humans, while others have side effects on the organs due to varying amounts of toxic matter present in such plants. The use of herbs requires good knowledge of toxicity dosage, purity of suitable extraction solvent, and adverse effects[3]. The dried fruits and seeds of *Monodora myristica* and *Xylopia aethiopica* are commonly used as spices in some West African cuisines to improve flavor and provide new taste and are also employed as therapy for the treatment of malaria, uterine fibroid, constipation, amenorrhea, headaches, sores and also as stimulants. They have been reported to possess antimicrobial, antioxidant, and hypocholesterolemic properties among others[4]. *Monodora myristica*, popularly called African Nutmeg, is a perennial plant of the *Annonacae* or custard apple of flowering plants[5]. *Monodora myristica* is a widespread and attractive small tree with decorative flowers, widely distributed from Africa to Asia. Inside its fruits are numerous oblong pale brown seeds which are usually 1.5cm long and are embedded in a white sweet smelling pulp[6]. Moreover, the bark and leaves are used in the treatment of various ailments in the African traditional medicines[7]. The spice has been reported to possess good antioxidant properties due to the presence of some flavonoids and phenol in its seeds[8]. They also contain bioactive substances that exhibit health-beneficial effects, including stimulation of the cardiac and circulatory system, anti-inflammatory, antispasmodic, diuretic, antihypertensive, cholesterol-lowering, antioxidant, anti-diabetic, hepatoprotective, antibacterial and antifungal activities. Most times, the crude extract of the seeds and fruits has been used in

combination with *Xylopia aethiopica* in the treatment of some ailments in traditional medicine[5].

Xylopia aethiopica, commonly called Negro Pepper, African pepper, and spice tree, is an evergreen, aromatic tree of the Annonaceae family that can grow up to 15-30m high[4]. It is widely spread in most African countries like the moist forests of Senegal, Ethiopia, Angola, Zimbabwe, and Mozambique[9]. The seeds of *Xylopia aethiopica* have an aromatic pungent taste and dried fruits are important as flavorings in the preparation of local soups in West Africa[10]. A study of the fruits of *Xylopia aethiopica* has shown that it has different biological activities including analgesia, vasorelaxant, and anti-inflammatory effects in rodents[11].

Figure 1: Matured *Xylopia aethiopica* ripe fruits

Figure 2: *Monodora myristica* seeds

The pancreas is a vital endocrine-exocrine organ of the digestive system that produces several hormones and enzymes that help in the digestion of carbohydrates, fats, and proteins. In humans, the pancreas is located in the abdominal cavity behind the stomach[12]. As an endocrine gland, it contains cells that produce several important hormones, which include insulin, glucagon, somatostatin, and pancreatic polypeptide, all of which circulate into the blood. The pancreas being part of the digestive system secretes pancreatic juice containing bicarbonate to neutralize the acidity of chyme moving in from the stomach, as well as enzymes that assist digestion and absorption of nutrients in the small intestine[3]. Therefore, any change in the function of the organ may directly affect the physiological function of the body[12].

Although *Monodora myristica* and *Xylopia aethiopica* are used as spices, safety is not guaranteed as to the doses and amount consumed. Although a notable number of in vitro and animal studies have been conducted to confirm their therapeutic uses, not much has been done on the evaluation of their effect on the histological and morphological changes in the structure of the pancreas. This study was carried out to assess the effect of aqueous seed extract of *monodora myristica* and *xylopia aethiopica* on the histology of the pancreas of adult male albino Wistar rats.

MATERIALS AND METHODS

Breeding of experimental animals

A total of twenty (20) adult male Wistar rats weighing about 160-180 g were used for the study. The rats were obtained and housed in the Animal House, College of Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria. The rats were allowed to acclimatize for one week under standard environmental conditions of 25°C – 27°C temperature in a 12-hour light and 12-hour dark cycle. The rats were fed with rat chow from Agro Feed Mill, Nigeria Ltd., and were given clean drinking water ad libitum.

Ethical consideration

Ethical approval with the number 222ANA2423 was obtained from the Faculty of Basic Medical Sciences, University of Calabar and all experiments involving animals were carried out in line with the guidelines of the committee on the Use and Care of laboratory animals.

Preparation of extract

Monodora myristica seeds and the dried fruits of *Xylopia aethiopica* were obtained from Effurun market, Delta State, Nigeria. The *Monodora myristica*

seeds were de-shelled and air dried while the fruits of *Xylopia aethiopica* were beaten to remove the seeds, and rinsed properly under tap water to remove dirt and debris. They were allowed to air dry and taken to the laboratory where they were further dried at 30^0C - 40^0C using a Memmert thermostatic oven model – (F-N: C508.0270). The samples were allowed to cool and then crushed into powdered form using a manual blender model – (F NO. 4 Quaker City Mill Philadelphia: PA.U.S.A.) and were packaged separately ready for the extraction process.

The grounded sample of *Monodora myristica* and *Xylopia aethiopica* were accurately weighed at about 500 g and 200 g respectively using a weighing balance model (AE-Adam) and each sample was soaked in 1500 ml of distilled water for 24 hours. The samples were filtered using a Whatman No. 1 filter paper. The samples were then concentrated into a syrupy residue at 400 C - 500 C using the thermostatic water bath model – (F.Nr:l508.0271). Each extract weighed about 4.8 g and 5.2 g respectively.

Experimental protocol and administration of extract

The animals were randomly assigned into four (4) groups of five (5) animals in each group labelled as groups A, B, C & D and housed in plastic cages with beddings. Group A served as the control group while groups B, C & D served as the experimental groups.

Group A rats received feed and water only, group B received 500 mg/kg of aqueous extract of *Monodora myristica*, group C received 500 mg/kg of *Xylopia aethiopica*, group D received a combined extract of 500 mg/kg of *Monodora myristica* and 500 mg/kg of *Xylopia aethiopica* extracts orally for eight weeks daily. At the end of the experiment, the rats were sacrificed using ketamine, the pancreas was incised, fixed in 10% formal saline, processed, and stained using hematoxylin and eosin staining techniques.

RESULTS

Following Haematoxylin and Eosin staining, the histological section of the pancreas in Group A showed normal histological features. The pancreas parenchyma presents the exocrine and endocrine parts. The exocrine part of the pancreas revealed normal pancreatic acini, interlobular ducts (ILD), intercalated ducts (ICD), secretory acinar cells (AC) with eosinophilic zymogen granules (ZG), and basophilic cytoplasm (C). A cluster of loosely packed pale-staining islet cells of Langerhans α- and β-cells with eosinophilic cytoplasm were seen with blood capillaries (BC). Sections of Group B which received 500 mg/kg of aqueous extract of *Monodora myristica,* showed normal pancreas parenchyma similar to the control group as interlobular ducts. acinar cells (AC) with basophilic cytoplasm were seen. The Langerhans islet was seen with loosely packed pale-staining α- and β-cells. The section presented hypertrophy of acini cells and congested blood vessels (BV).

Group C which received 500 mg/kg of aqueous extract of *Xylopia aethiopica,* showed normal pancreas parenchyma also similar to Group A, acini with prominent secretory acinar cells and basophilic cytoplasm. The section also presented the presence of pyknotic nuclei and vacuolated cytoplasm in the islet of Langerhans. Group D which received 500 mg/kg of *Monodora myristica* and 500 mg/kg of *Xylopia aethiopica* combined aqueous extract, showed normal pancreas architecture similar to that of the control group. Also, prominent acini are seen with increased zymogens' granules within the acinar cells. The Langerhans islets were seen with loosely packed pale-staining cells. The interlobular ducts were found with secretions and periductal fibrosis occurring in the ducts. The section also showed congested blood vessels.

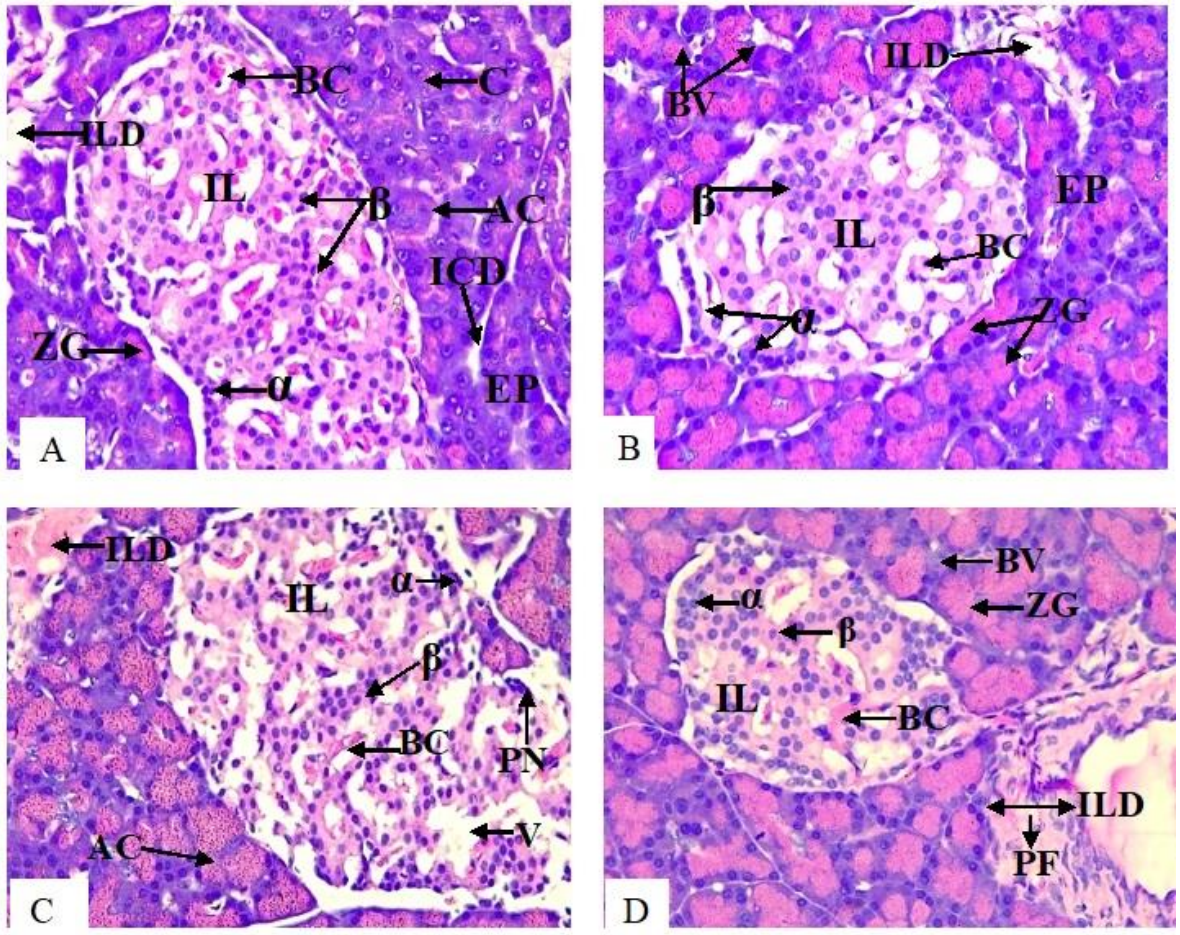

Figure 3: (A) Photomicrograph of the control showing normal pancreatic acini (B) Photomicrograph of group B showing hypertrophy of acini cells and congested blood vessels (C) Photomicrograph of group C showing presence of pyknotic nuclei in the islet of Langerhans (D) photomicrograph of group D showing secretions in the interlobular ducts, congested blood vessels and periductal fibrosis.

β- Beta cells, α- Alpha cells, ZG- Zymogen granules, ILD- Interlobar Duct, C- Basophilic cytoplasm, ICD- Intercalated Duct, BC- Blood Capillary, BV- Blood vessels, AC- Acinar cells, PF- Periductal fibrosis, V- vacuolated cytoplasm, PN- Pynotic nuclei. Hematoxylin and Eosin, x400

DISCUSSION

In this research work, the oral administration of aqueous seed extracts of *Monodora myristica* and *Xylopia aethiopica* to adult male Wistar rats shows the basic structure of the pancreas was similar in all the groups. This result agrees with similar observations made by several authors including Udo Affah *et al*[13] and Baset *et al*[14] as they concluded that normal rats showed a typically healthy pancreas architecture in the exocrine and endocrine (pancreatic islets of Langerhans) parts.

The histology section of the rats administered *Monodora myristica* extract showed varying findings which included congested blood vessels and acinar cell hypertrophy which was manifested by enlarged acinar with an increased number of zymogen granules in the apical part of the acinar cells when compared to the control group. This may be due to increased hormonal stimulation by the extract. These findings are similar to a study carried out by Myer *et al*[15]. The increased number of zymogen granules, when compared to the control group may be due to a disruption in the normal digestive activities of the pancreas as the granules do not release their contents properly into the ducts. This finding is similar to that of Flack[16] which suggested that these characteristics may be associated with mild acute pancreatitis. This result suggests that *Monodora myristica* may have caused a mild pancreatic injury that led to this response from the acinar cells and blood vessels.

The histological section of the rats administered aqueous fruit extract of the *Xylopia aethiopica* also shows normal pancreas structure. However, it was

observed that there was the presence of pyknotic nuclei and vacuolated cytoplasm within the endocrine pancreas when compared with the control group. The presence of the pyknotic nuclei in the islets of Langerhans indicates that irreversible damage has occurred leading to shrinkage of the islet cells as a result of necrosis or apoptosis. This observation is similar to Udo-affah *et al*.[13] who studied the effect of the ethanolic ginger extract on the integrity of the islet cells and suggested that the disintegration of the islets cells can lead to several illnesses as the cells demonstrated pyknotic nuclei in the Langerhans Islets. The vacuolated cytoplasm could indicate loss of nuclei materials or islet cells due to the pyknosis that occurred in the Langerhans islet. *Xylopia aethiopica* has been reported to improve the morphology of the islets and stimulate the recovery of beta cells in the pancreas of diabetic rats[17]. However, the presence of histological cellular alterations in this section suggests that *Xylopia aethiopica* may have a cytotoxic effect on the morphology of the endocrine pancreas which may be dose or duration-dependent.

The experimental group which received a combined aqueous extract of *Xylopia aethiopica* and *Monodora myristica* presented the acinar cells to be hypertrophic when compared to the control group but is similar to that of group B. This could be a result of the high calcium content in the combined extracts or hormonal stimulation. This result agrees with an assessment carried out by authors who reported that prolonged calcium (ca2+) entry through store-operated channels (SOCs) in the acinar cells could contribute to pancreatitis[18]. The cells of islets of Langerhans showed improved morphology when compared to the group administered solely *Xylopia aethiopica* extracts showing an increased number of islet cells and reduced signs of cytoplasmic vacuolation. This result is consistent with the findings of Ofusori *et al*.[17] and Mohammed *et al*.[19]. This result also presented periductal fibrosis in the interlobular duct. This is similar to the findings of Kloppel *et al*.[20] who reported that periductal fibrosis could be caused by processes such as necrosis/apoptosis and/or ductal obstruction as well as injury to the interstitial mesenchymal cells, interlobular duct, or the acinar cells. Periductal fibrosis is observed as a thickening surrounding the duct's epithelium giving an impression of a thickened duct wall. This may be caused by excessive deposition of extracellular matrixes of collagen and fibronectin in the pancreatic tissue as a result of repeated injury often seen in cases of chronic pancreatic disease[21]. This result indicates that the combined extract of *Xylopia aethiopica* and *Monodora myristica* may have an adverse effect on the histology of the pancreas.

The possible cause of cellular alterations in the pancreas architecture may *also* be associated with prolonged administration, dosage, or long-term exposure to the active phytochemical constituents such as alkaloids, flavonoids, saponins, etc. in *Xylopia aethiopica* and *Monodora myristica* especially in the experimental group C and D as also agreed by Obhakhan *et al*.[22], Enabulele *et al*.[23] and Ogbagu *et al*.[24]. Therefore, effect seen may be said to be dose-dependent or duration-dependent.

Conclusion

From the histological assessment carried out, it may be deduced that *Monodora myristica* and *Xylopia aethiopica* may have an adverse effect on the cytoarchitecture of the pancreas particularly the Islets of Langerhans.

Conflict of interest

None declared.

Authors' contribution

Anyanimeh J. Ukoh: Laboratory experiment, Writing-original draft, review and editing, Ejiro Ederademu: Conceptualization, Methodology, Experimental Design, Laboratory experiment, Affiong E. Ekanem: Writing- review and editing, Mokutima A. Eluwa: Supervision, Writing- review and editing.

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