

Histomorphological Assessment of The Effect of *Aristolochia Ringens* on the Pancreas of Albino Wistar Rats

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Abstract— The use of *Aristolochia ringens* in Nigeria and other countries in the world for the treatment of several ailments have been reported but not without consequences such as nephritis, uroepithelial tumorigenesis, urothelial cancer and liver cancer. This plant has an active compound called aristolochic acid which is a potent carcinogen. In this work, histomorphological assessment of aqueous leaf extract of *Aristolochia ringens* was studied. Twenty adult male albino wistar rats were used for the study. The animals were divided into four groups of five animals each. Group one was the control group and was given 5 ml of distilled water. Groups 2,3,4 were the experimental groups, and were given 47.43 mg, 94.87 mg, and 142.30 mg of *Aristolochia ringens* extract per kilogram body weight respectively for 21 days. On the 22nd day animals were euthanized using chloroform inhalation. Pancreas were harvested and fixed in 10% buffered formalin; then processed and stained for histological studies using haematoxylin and eosin stain. Results obtained showed no observable distortion in the histology of the pancreas. Hence, oral administration of aqueous leaf extract of *Aristolochia ringens* at these dosages for 21 days caused no observable distortion in the cells and tissues of the pancreas.

Index Terms— *Aristolochia ringens*, Aristolochic acid, Histology, Pancreas.

I. INTRODUCTION

In recent years, the traditional application of natural compounds of plant origin has been receiving a lot of attention as an alternative source of remedy for the treatment of diseases. This has led to the increase in laboratory (*in-vitro*) research into herbal medicine to establish their efficacy and their therapeutic applications [1].

Aristolochia ringens is a glabrous bushy climber native of tropical America, introduced to most West African countries as a garden ornamental, and has become naturalized in roadside bush in Sierra Leone, Ghana, Nigeria [2] and DR Congo [3]. In Nigeria, it is mostly found in the north, west, and south – south [4]. The plant is commonly called “Dutchman’s pipe” and “Snake work” but local names in Nigeria include “UbongEdop” (Ibibio, South-South Nigeria) “Ako-igun” (Yoruba, Southwest Nigeria) and “Dumandutsee” (Hausa, Northern Nigeria).



Image showing *Aristolochia Ringens* [5]

Preparations of the leaves, roots, and whole plant have been reported to be used traditionally in Nigeria and other countries for the treatment of diverse ailments. In South America, the plant is used for the treatment of snakebites, fever, ulcers and colon inflammation while the root of the plant is used in Senegal as an antidote for snakebites [6]. [7] Stated that the root of the plant is used in Southwest Nigeria for the treatment of asthma, while [8] reported its use for the treatment of hemorrhoids. The decoction/infusion of the root of the plant is also used as an antidiabetic [9], anti-inflammatory [10] and antitrypanosomal [11]. In Ibibio land (South-South Nigeria), the leaves of the plant alongside with its stock are used by the traditional bone setters for bone setting and anti-inflammatory activities.

The constituents of the plant include the flavonoids, alkaloids, tannin and aristolochic acids. The most active constituents of the plant are the aristolochic acids. Aristolochic acids are a family of carcinogenic, mutagenic, and nephrotoxic phytochemicals commonly found in the birthwort (*Aristolochiaceae*) family of plants [12].

Although this plant has reported benefits, it has also been implicated in Nephritis, urothelial cancer [13], uroepithelial tumorigenesis and liver cancer [14].

The pancreas is a secretory structure with an internal

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hormonal role (endocrine) and an external digestive role (exocrine). In humans, it is located in the abdominal cavity behind the stomach. The endocrine part is composed of hormonal tissue distributed along the pancreas in discrete units called islets of Langerhans; producing several important hormones, including insulin, glucagon, somatostatin, and pancreatic polypeptide, all of which circulate in the blood [15]. Islets of Langerhans have a well-established structure and form density routes through the exocrine tissue. The exocrine part has two main ducts, the main pancreatic duct and the accessory pancreatic duct. These drain enzymes through the ampulla of Vater into the duodenum, pancreatic islet [15].

The role of pancreas in Sugar control and metabolism was a motivation to investigating the consequence of chronic and high dose administration of *Aristolochiaringsen* on the histology of pancreas in adult albino wistar rats.

II. MATERIALS AND METHOD

Twenty adult male albino wistar rats weighing between 100g-185g were used for this study, these animals were gotten from faculty of Pharmacy University of Uyo and were assigned into four groups of five animals each, group one served as control while groups two, three, and four served as experimental. The animals were housed in a cross ventilated cage and allowed free access to food and water. Good hygiene was maintained by constant cleaning and removal of faeces and spilled food from the cage daily.

Fresh leaves of *Aristolochiaringsen* was gotten from a local farm in Ayadehe in Itu Local Government Area, Akwalbom State, Nigeria. The plant leaves were identified and authenticated by the Department of Botany and Ecological Science, University of Uyo. Fresh leaves of *Aristolochiaringsen* were macerated and soaked in 800 millimetres of water overnight. The macerated leaves were filtered and the aqueous extract was dried in a water bath, and the dried extract was stored in a refrigerator.

The mean lethal dose, (LD_{50}) of the extract was calculated to establish the dosage of the extract that will elicit expected pharmaceutical effect on the experimental animals without being toxic to the system of the animal.

$$\text{Calculation of } LD_{50} = \frac{\text{weight of animals (g)} \times 1000 \text{ mg/kg}}{1000 \times 50 \text{ mg/kg}}$$

$$LD_{50} = \sqrt{AB}$$

A = 450 mg/kg (maximum dose that produce 0% mortality)

B = 250 mg/kg (minimum dose that produce 100% mortality)

$$\text{Therefore } LD_{50} = \sqrt{450 \times 250} \\ = 474.34 \text{ mg/kg}$$

The animals in the four groups were weighed before commencement of administration of extract and weighed again at one week interval till the end of the administration. The aqueous leaf extract of *Aristolochiaringsen* were given to groups two to four in the dose of 47.42 mg/kg, 94.87 mg/kg and 142.30 mg/kg respectively, according to their body weight for twenty one days. Group one (control group) was given 5ml of distilled water for 21 days.

On the day 22, animal were euthanize using chloroform inhalation. Pancreas were harvested and fixed in 10% buffered formalin for seven days; then processed and stained for histological studies using haematoxylin and eosin stain.

Tissues were obtained from each group trimmed into 6mm and placed in 70% alcohol overnight. The tissues were subjected to dehydration in two changes of 70%, 90%, 95% and absolute alcohol for 45mins each, cleared in two changes of xylene for 45 minutes each; then embedded in molten paraffin at 60 degree Celsius for infiltration, and allowed to dry for sectioning. Sections were deparaffinised using two xylene washes of two minutes each followed by dehydration; then rinsed twice in 100%, 95%, 70% alcohol for five minutes each and finally rinsed in tap water. The sections were stained in haematoxylin for 10 minutes, washed briefly in running tap water differentiated in 1% acid alcohol washed in running tap water for fifteen minutes and blued in Scott's water chlorine. Sections were counter stained in eosin for five minutes, washed in tap water, dehydrated in 95% and absolute alcohol cleared and mounted using DPX.

III. RESULTS

Photomicrographs presented in this study were obtained from routine histological stains using hematoxylin and eosin staining at X100 and X400 magnification.

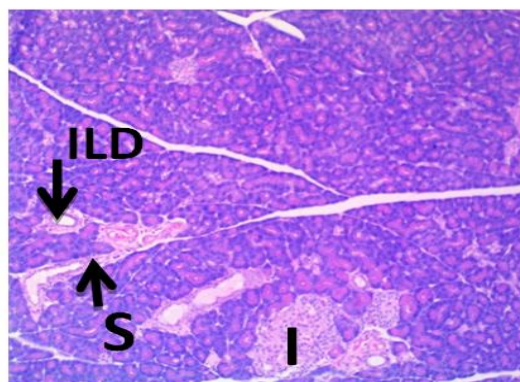


Plate i: photomicrographs of pancreas given distilled water; H&E. X100. I: islet of Langerhans; ILD: interlobular duct; S: septum.

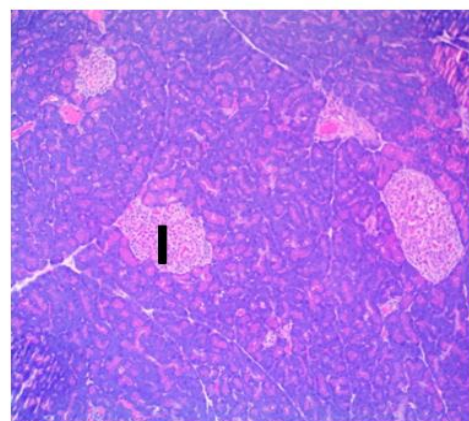


Plate ii: photomicrographs of pancreas treated with 47.43 mg/kg of *Aristolochiaringsen* leaf extract; H&E. X100. I: islet of Langerhans.

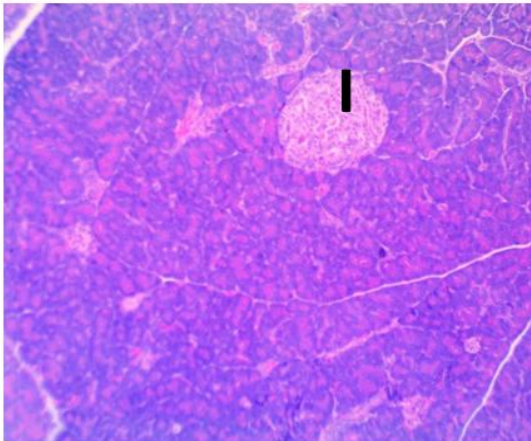


Plate iii: photomicrographs of pancreas treated with 94.87 mg/kg of *Aristolochiaringsens* leaf extract; H&E. X100. I: islet of Langerhans.

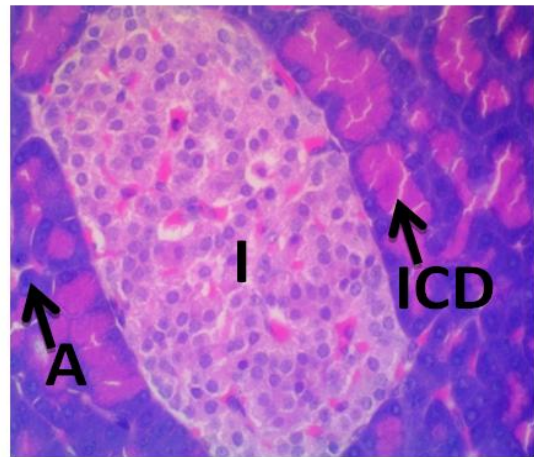


Plate vi: photomicrographs of pancreas treated with 47.43 mg/kg of *Aristolochiaringsens* leaf extract; H&E. X400. I: islet of Langerhans; A: acini cell; ICD: intercalated duct.

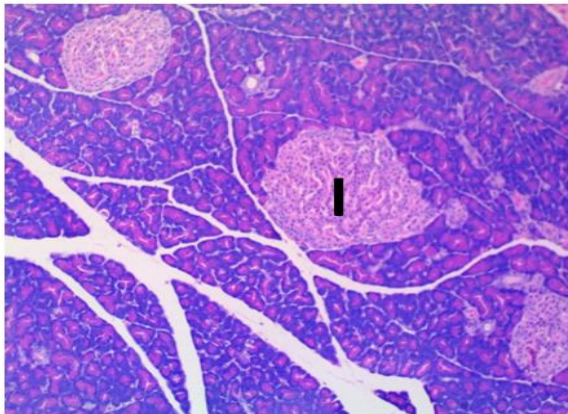


Plate iv: photomicrographs of pancreas treated with 142.30 mg/kg of *Aristolochiaringsens* leaf extract; H&E. X100. I: islet of Langerhans.

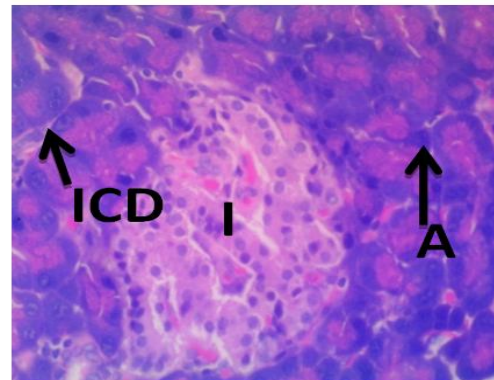


Plate vii: photomicrographs of pancreas treated with 94.87 mg/kg of *Aristolochiaringsens* leaf extract; H&E. X400. I: islet of Langerhans; A: acini cell; ICD: intercalated duct.

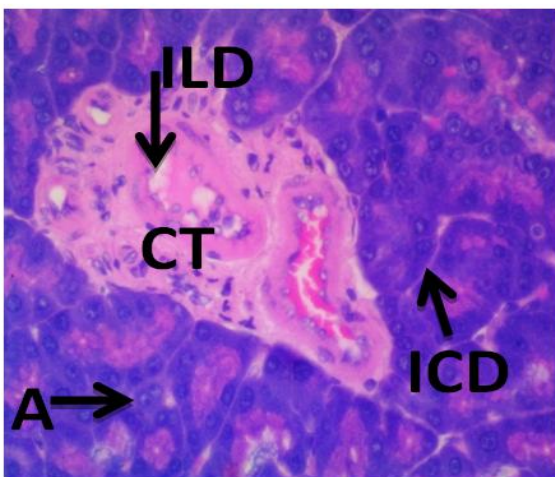


Plate v: photomicrographs of pancreas given distilled water; H&E. X400. A: acini cell; ICD: intercalated duct; ILD: interlobular duct; CT: connective tissue.

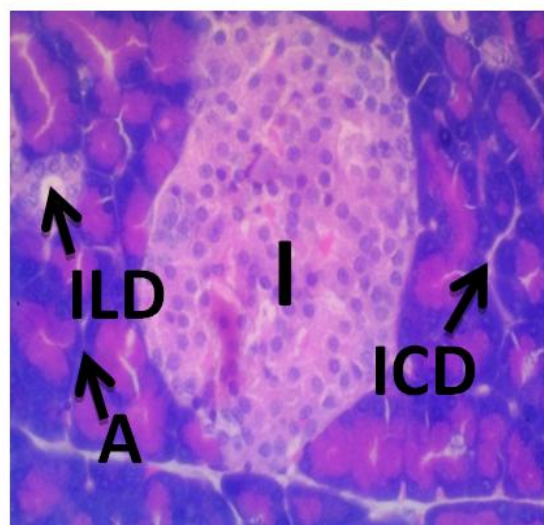


Plate viii: photomicrographs of pancreas treated with 142.30 mg/kg of *Aristolochiaringsens* leaf extract; H&E. X400. I: islet of Langerhans; A: acini cell; ICD: intercalated duct; ILD: interlobular duct.

IV. DISCUSSION

Histological evaluation on photomicrographs of pancreas of control and treatment group was carried out.

The control group administered with 5ml of distilled water showed normal cytoarchitecture of the pancreas with normal histological appearance of Islet of Langerhans, acini cells, intercalated duct and interlobular duct.

Results from treatment groups 2-4, treated with 45.73 mg/kg, 94.87mg/kg, 142.30 mg/kg respectively were also evaluated. This investigation showed no observable distortion in the cytoarchitecture of the pancreas of treatment groups when compared with the control group. There was no observable difference in the histological appearance of islets of Langerhans, acini cells, intercalated and interlobular ducts seen in treatment groups when compared with the control group. We also observed no difference in histological appearance when treatment groups were compared with each other. This therefore implies that the administration of *Aristolochiarogens* extract at dosages 47.43 mg/kg, 94.87 mg/kg and 142.30 mg/kg for 21 days caused no observable distortion in the cells and tissues of the pancreas.

V. CONCLUSION

Oral administration of 47.43 mg/kg, 94.87 mg/kg, and 142.30 mg/kg of aqueous leaf extract of *Aristolochiarogens* for 21 days caused no observable distortion in the cells and tissues of the pancreas.

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