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## **Histopathological Effect of Crude Extract of *Piper Guineense* (Uziza) Leaves on the Liver of Wistar Rats and on Pseudofolliculitis Barbae (After Shave Rash) in Human**

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### **Abstract**

The use of plant medicine dates back to ancient times, the nearly all year round availability and the relatively cheaper cost of medicinal plants in sub Saharan Africa makes them more attractive as therapeutic agents when compared to the orthodox medicine. The use of herbal medicine is on the increase globally, hence the increased interest on research on herbal formulations and preparations. Some of these plants are used as condiments and ingredients in food thus referred to as local spices. One of such plants used as a local spice in Nigeria is *Piper guineense* traditionally called 'Uziza' amongst the Igbo tribe, 'Iyere' amongst the Yoruba tribe, 'Etinkene' amongst the Efik and Ibibio tribe and 'Ebe-ahinhi akpoke' amongst the Edo tribe. The leaves and seeds of *Piper guineense* were initially consumed in the Southern part of Nigeria. The aim of this research is to detect and demonstrate any histological effect of *Piper guineense* leaves on the liver of Wistar rats and on after shave rash in human. During the acclimatization period which lasted for two (2) weeks, three rats died due loss of appetite, emaciation, weakness, dislocation of neck with head leaning to the right, consistent rolling over and vigorous twirling in circular motion during carriage. After two weeks of acclimatization, the rats weighed; The *Piper guineense* leaves was able to effect total removal of the rash when used on the consented individual within day 3 and day 4 of usage. *Piper guineense* leaves when taken

into reasonably consideration causes no histological changes of the liver tissues, hence its use should be encouraged both for consumption and in treatment of pseudofolliculitis Barbae (after shave rash).

**Keywords:** Liver, *Piper guineense*, wistar rat, Pseudofolliculitis Barbae

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## 1. Introduction

The use of plant medicine dates back to ancient times, the nearly all year round availability and the relatively cheaper cost of medicinal plants in sub Saharan Africa makes them more attractive as therapeutic agents when compared to the orthodox medicine [1-2]. The use of herbal medicine is on the increase globally [3-4], hence the increased interest on research on herbal formulations and preparations [5]. Some of these plants are used as condiments and ingredients in food thus referred to as local spices. One of such plants used as a local spice in Nigeria is *Piper guineense* traditionally called 'Uziza' amongst the Igbo tribe, 'Iyere' amongst the Yoruba tribe, 'Etinkene' amongst the Efik and Ibibio tribe and 'Ebe-ahinhi akpoke' amongst the Edo tribe. The leaves and seeds of *Piper guineense* were initially consumed in the Southern part of Nigeria but overtime its consumption has spread across the entire country even into neighboring countries like Benin republic, Ghana, Liberia, Cameroun amongst others [6-7] Also, its incorporation in intercontinental dishes and its high demand by Nigerians in foreign countries has promoted its use in countries far and wide. An assessment of the effect of *Piper guineense* consumption on various human organs is essential to ascertain merits and detect demerits if any in order to avert health conditions that may arise overtime due to continual use. An organ of particular interest is the Liver; the body's largest compound organ weighing 1.5kg in adult humans. It lies in the right upper quadrant of the abdomen and is completely protected by the thoracic rib cage. It is the principal metabolic organ and massive detoxification center for alcohol, drugs and other chemical substances, thus is directly influenced overtime by the toxicity of substances assimilated. The function of the liver as a detoxifying and clearance organ harbors the danger that the substances that should be degraded and/or eliminated eventually lead to tissue damage [8]. Nature has been a source of medicinal treatment for

thousands of years and plant-based systems continue to play an essential role in the primary health care of underdeveloped and developing countries. Plants have formed the basis of traditional medicine system that has been the way of life for thousands of years [9]. The liver is a vital organ present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemicals necessary for digestion. The liver is necessary for survival and there is currently no way to compensate for the absence of liver function in the long term, although new liver dialysis techniques can be used in the short term. *Piper guineense* has been implicated as the cause of macrovesicular steatosis, disrupted liver cyto-architecture and cell necrosis when administered in high doses, thus implying the possibility of toxic side effects when used at certain doses consistently over a long period of time. The aim of this research is to detect and demonstrate any histological effect of *Piper guineense* leaves on the liver of Wistar rats and investigate the curative effect of crude extract of *Piper guineense* leaves on Pseudofolliculitis barbae (after shave rash).

## 2. Materials and Methods

This research was performed in Department of Medical Laboratory Science, University of Benin, Nigeria. Thirty male Wistar rats of average body weight (125-145g) were purchased from the Department of Physiology, University of Benin, Nigeria. They were housed in a well ventilated room with a temperature of 25-27 °C under the natural light and dark cycle. Fresh *Piper guineense* leaves were purchased from University of Benin garden. The *Piper guineense* leaves were sun dried for fourteen days to ensure proper removal of water content after which it was grinded into powder using an attrition mill (a locally fabricated mill). The ground leaves were stored in a tightly covered

plastic container to prevent the entrance of moisture, part of the grounded powder was taken to constitute a paste. The thirty male Wistar rats were fed with standard laboratory diet and water ad libitum for an acclimatization period of fourteen days. After this period, the animals were divided into four groups, one control (iv) and three experimental groups (I, II,III,). The control group was given clean water and pelletised growers feed while the experimental group I, II, III, and IV were fed increasing concentrations of *Piper guineense* leaves; 0.009g, 1.509g, 3.009g, 4.509g, and 6.009g per rat per serving. The rats were fed twice daily (morning and evening) for fourteen days. The rat cage was cleaned every thirty two hours and all experimentations were carried out following the guideline for the care and use of laboratory animals obtained from the Institution's animal ethical committee. The Nutritional Composition of the Pelletised growers feed is; Crude Protein (14%), Fat (7%), Crude Fibre (10%), Calcium (1%), Available Phosphorus (0.35%), Metabolisable energy (2550kcal/kg). LD<sub>50</sub> of *Piper guineense* is 100mg/kg.

Average rat weight is 175g; therefore, LD<sub>50</sub> is 17.5mg/175g daily, 8.75mg/175g per serving.

From the feeding pattern observed during acclimatization, 5 rats consume 65g of feed per serving indicating that 1 rat consumes 13g of feed.

From personal findings, it was discovered that a 70kg human consumes 3.75g of Uziza per serving thus providing a baseline for *Piper guineense* constitution in the experiment.

Twelve hours after the last feeding, the animals were anesthetized with chloroform vapor and dissected. The harvested liver was carefully dissected out, trimmed of all fat and connective tissue and blotted dry to remove any blood. The tissues were then fixed in 10% formal saline and transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. The tissues were then passed through three changes of molten paraffin wax at one-hour interval, after which the tissues were embedded in

paraffin wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were properly orientated. Serial sections of 5µm thick were obtained from a solid block of tissue, fixed on clean albuminized slides to enhance adhesion and later stained using the Haematoxylin and Eosin staining technique which was performed by; Dewaxing the sections in Xylene and Hydrating through descending grades of alcohol (Absolute, 90%, 80%, 70%) and finally into water, Staining for ten minutes in Ehrlich Haematoxylin and rinsing afterwards in water, Differentiating briefly in 1% acid alcohol and rinsing afterwards in water, Bluing for ten minutes in Scot tap water, Counterstaining for two minutes in 1% aqueous eosin and rinsing afterwards in water, Dehydrating through ascending grades of alcohol (70%, 80%, 90% and Absolute), Clearing in two changes of xylene and air drying, Mounting in synthetic resin medium (DPX) and Examining with light microscope using x10 and x40 objective magnification. The initial and final weights of the rats and weight of the liver were measured using a weighing balance. Records of the histological results were obtained by photomicrography using digital photomicrographic microscope in the Department of Medical Laboratory Science, University of Benin, Benin city, Edo state. The constituted paste was used on consented individuals and photographs were taken.

### 3. Results

The result from this work shows clearly that *Piper guineense* leaves has no histopathological, inflammatory or cellular damage on the liver cells of the wistar rats as shown in figure 2 and3 below. The *Piper guineense* leaves was able to effect total removal of the rash when used on the consented individual within day 3 and day 4 of usage as shown in figure 3 and 4 below.

### 4. Conclusion

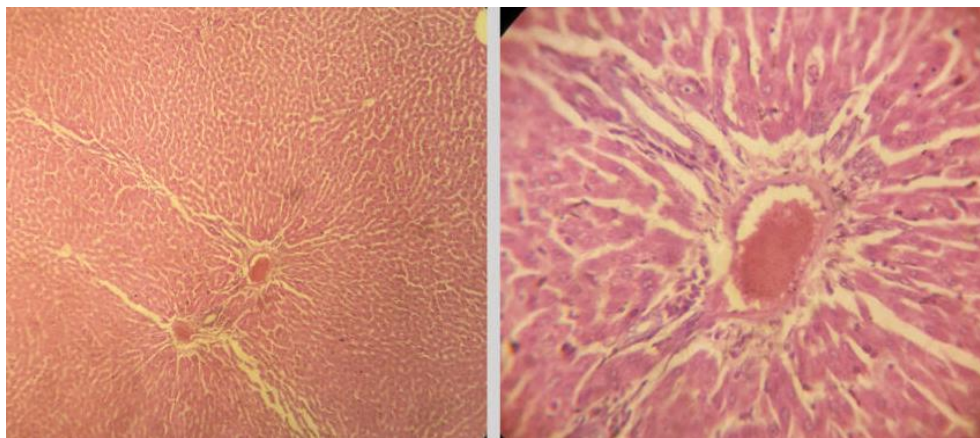
*Piper guineense* leaves when taken into reasonably consideration causes no histological changes of the liver tissues, hence its use should be encouraged both for consumption and in treatment of pseudofolliculitis Barbae (after shave rash).

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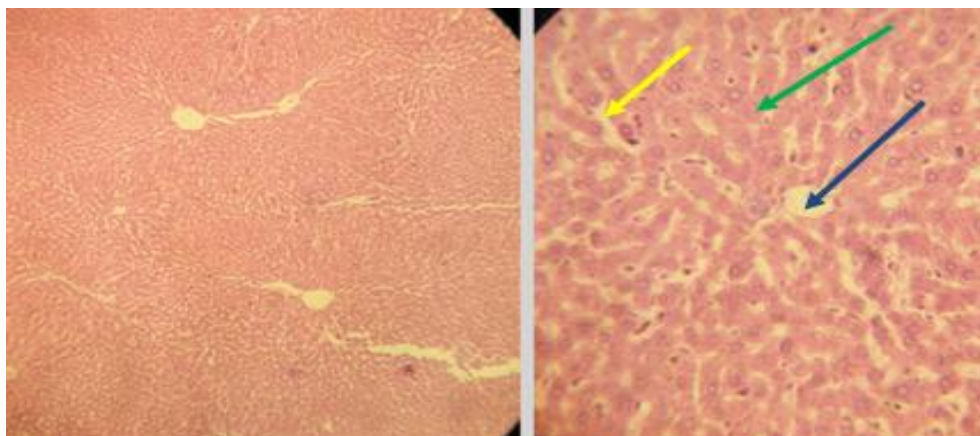
## 5. Recommendation

There was improved alertness and appetite observed in all the experimental Wistar rats especially those in the groups fed with the highest concentration of

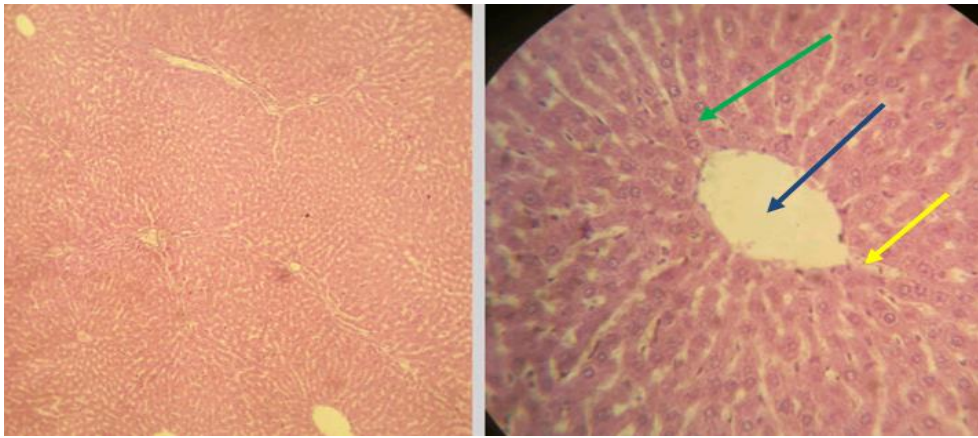
*Piper guineense* leaves and this is also contrary to the report by Ebeye *et al.* (2007) stating reduced agility and loss of appetite in the rats fed with higher concentrations of the *Piper guineense* seed extract[2]. This variance could be due to the duration of the experiment which resulted in prolonged feeding and apparent lack of interest in the feed by the experimental animals. The extract which of used to constitute a paste was used on after shave rash and reduction was seen after 2 to three days of usage.



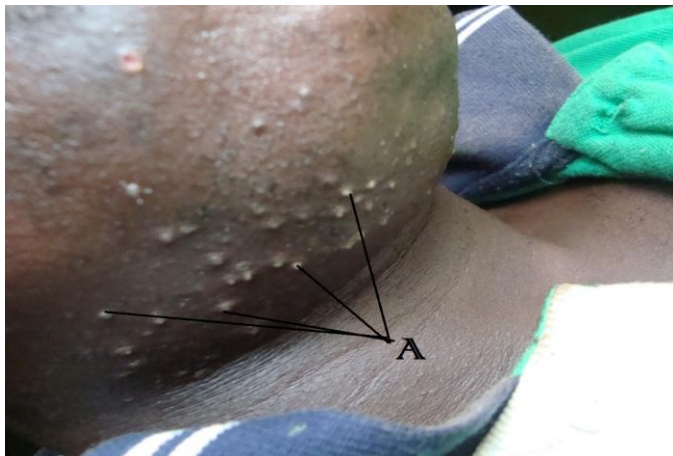
**Figure 1.** H&E Photomicrograph of Control liver at X400 showing a central vein with red blood cells (Blue arrow), hepatocytes (Green) and sinusoids (Yellow). The architecture is normal with normal cells. There are no inflammatory cells



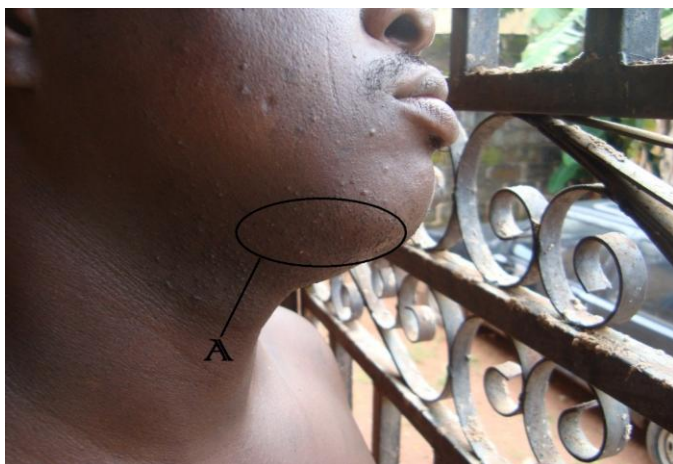
**Figure 2.** H&E Photomicrograph of Group I liver X400 objective magnification. There are no inflammatory cells. There is no evidence of congestion or necrosis. The hepatocytes (Green) and sinusoids (Yellow) are also normal. The central vein (Blue arrow) is also normal



**Figure 3.** H&E Photomicrograph of Group II liver at X400 objective magnification. There are no inflammatory cells. There is no evidence of congestion or necrosis. The hepatocytes (Green) and sinusoids (Yellow) are also normal. The central vein (Blue arrow) is also normal



**Figure 4.** Before use of The *Piper guineense* leaves on Pseudofolliculitis Barbae



**Figure 5.** After use of The *Piper guineense* leaves on Pseudofolliculitis Barbae

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