



ISSN: 1697-090X

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# HISTOLOGICAL EFFECTS OF CHRONIC ADMINISTRATION OF AQUEOUS EXTRACT OF *PHYLLANTHUS AMARUS* ON THE STOMACH AND THE DUODENUM OF WISTAR RATS

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Rev Electron Biomed / Electron J Biomed 2011;1:9-13.

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## ABSTRACT

**Background:** *Phyllanthus amarus* is a herbal agent commonly used for the treatment of several diseases including gastric ones. A systematic study about its toxic effects when chronically and excessively administered is still lacking.

**Aim:** This study investigates the histological effects of chronic administration of the herb on the stomach and the duodenum of adult Wistar rats of both sexes.

**Material and methods:** Rats, with average weight of 200g, were divided into three groups of eight animals each: A (*P amarus* at a single dose of 400mg/kg body weight daily for thirty days through an orogastric tube); B (*P. amarus* at a single dose of 800mg/kg body weight daily through the same route and period), and C (controls, receiving equal volume of distilled water in identical conditions to A and B). Rats were fed with grower's mash and received water *ad libitum*. Animals were euthanized by cervical dislocation on the thirty-one day of the experiment and the stomach and the duodenum were immediately dissected out and fixed in 10% formaldehyde- saline for routine histological techniques. **Results:** Rats in the treated groups showed some distortions in the epithelial cells of the stomach and the duodenum and signs of inflammation, when compared with controls.

**Conclusion:** This report provides evidence that ethnomedicinal practice involving chronic administration of *Phyllanthus amarus* at high doses has potential adverse histological effect in determined digestive organs. It is likely that the herb may be contraindicated in gastro-ulcer patients. This warrants further studies to establish or rule out any untoward side-effect on bowel response and/or digestive dysfunctions.

**Keywords:** Antioxidant toxicity, digestive tract, ethnomedicinal practice, histological effects, *Phyllanthus amarus*

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**RESUMEN:** EFECTOS HISTOLOGICOS DE LA ADMINISTRACIÓN A LARGO PLAZO DEL EXTRACTO ACUOSO DE *PHYLLANTHUS AMARUS* SOBRE EL ESTÓMAGO Y EL DUODENO DE RATAS WISTAR

**Antecedentes:** *Phyllanthus amarus* es un agente a base de hierbas de uso común para el tratamiento de varias enfermedades incluyendo los trastornos gástricos. Aun está pendiente un estudio sistemático sobre sus efectos tóxicos cuando se administran en altas dosis por largo periodo de tiempo.

**Objetivo:** El objeto de este estudio es investigar los efectos de la administración crónica de esta hierba sobre la histología del estómago y duodeno de ratas Wistar adultas de ambos sexos.

**Material y métodos:** Las ratas, con un peso promedio de 200 g, se dividieron en tres grupos de ocho animales cada uno. El grupo A recibió *P. amarus* en dosis única de 400 mg/kg de peso corporal al día durante treinta días mediante sonda gástrica, el grupo B recibió *P. amarus* en una sola dosis de 800mg/kg de peso corporal al día con el mismo procedimiento y período, y el grupo C fue de ratas control, que recibió el mismo volumen de agua destilada en idénticas condiciones que A y B. Las ratas fueron alimentadas con pienso especial para ratas y agua *ad libitum*. Los animales fueron sacrificados por dislocación cervical en el día treinta y uno del experimento y el estómago y el duodeno fueron disecados e inmediatamente fijados en formol al 10% en solución salina para técnicas histológicas de rutina. Resultados: En los grupos tratados, las ratas mostraron algunas distorsiones en las células epiteliales del estómago y el duodeno y signos de inflamación, en comparación con los controles.

**Conclusión:** Este trabajo present evidencias de que la práctica etnomedicinal de administración a largo plazo de *Phyllanthus amarus* en altas dosis tiene potencial efecto adverso histológico en determinados órganos digestivos. Es probable que esta hierba puede estar contraindicada en pacientes con úlcera gastrointestinal. Se requieren estudios adicionales para establecer o descartar efecto secundarios en la respuesta intestinal y / o trastornos digestivos.

**Palabras clave:** Toxicidad antioxidante. Tracto digestivo. Etnomedicina práctica. Cambios histológicos, *Phyllanthus amarus*

## INTRODUCTION

Herbal medicines are widely perceived by the public as being free from side effects. However, herbal therapies have not been effusively researched or standardized to enable clinical application<sup>1</sup>. Indeed, some of the herbal agents are unknown in the Western world and concerns regarding side effects in modern evidence-based medicine practice are major<sup>2</sup>. Hence, safety of herbal medicines is still an issue worldwide<sup>2-4</sup>.

*Phyllanthus amarus* (*P. amarus*) is an ethnobotanical plant that is distributed in almost all tropical countries and regions including America, India and Nigeria. It is a common weed, which grows well in moist, shady and sunny places. It is known by several other names such as carry-me-seed, chanca piedra, and quinine weed to mention a few<sup>5</sup>. *P. amarus* is believed to possess antioxidant, antiseptic, and stomachic properties among others, and it is used accordingly in many countries<sup>6-10</sup>. It is particularly used traditionally in the treatment of several bowel diseases including diarrhea, dysentery and ulcers<sup>5,10-11</sup>.

Plants contain hundreds of constituents and some of them may elicit toxic side effects. Phytochemically, *P. amarus* contains alkaloids, antioxidants (including flavonoids, phenols and polyphenols) and lignans<sup>5</sup>. All of these chemical components have their useful as well as toxic effects. The toxic effects of *P. amarus* has been suggested in literature<sup>12-13</sup>. However, histological perspectives are yet to be elaborated.

In mammals including humans and rats, the digestive tract is composed of tubular organs that are joined together sequentially from the mouth, esophagus, stomach, small intestine (including duodenum, ileum and jejunum) and large intestine to the anus. Along this continuum of the digestive tract, chemical digestion occurs minimally in the mouth, predominantly in stomach and duodenum, and is almost complete at arriving to the ileum. It is known that digestion of certain foods such as vegetables is often incomplete<sup>14</sup>. It is also known that while food is emptied from the mouth and oesophagus as quickly as it is swallowed, emptying of the stomach and small intestine in digestive process is relatively slow and not consciously controlled. Yet, it is varied for different types of food. For instance, proteins are emptied slower than carbohydrates<sup>15</sup>. The implication is that any toxic content of food may have very limited time to affect the mouth and oesophagus, the potential risk on the stomach or small intestine is impacted by the relative longer time the food stays in these two regions.

Therefore, the stomach and duodenum are logically the regions of the digestive tract that may be most affected by any toxic component of foods. Since *P. amarus* posses some potentially toxic phytochemicals<sup>5</sup>, it would be worthwhile to explore the effects of chronic administration of the herb on the microanatomy of the digestive tract by examining the stomach and duodenum.

## MATERIALS AND METHODS

**Animals and ethical concerns:** This study is part of a project on the histological effects of *P. amarus* research. Twenty-four adult Wistar rats of both sexes, with average weight of 200g, were equally and randomly assigned into three groups (n=8 each): two treatment groups [A] and [B] and one, untreated or control [C]. The School of Basic Medical Sciences, University of Benin granted approval before the work began.

The animal care and use ethics was in compliance to the Animal Holdings protocol overseen by the head of department through the Animal Holding unit. Rats were obtained and maintained in the Animal Holdings of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city, Edo State, Nigeria. The animals were fed with grower's mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo State, Nigeria and received water *ad libitum*.

**Preparation and administration of *P. amarus*:** The *P. amarus* leaves were obtained in Benin City. The leaves were cleaned, oven-dried at 50°C and macerated into dry powder. The powder was extracted with distilled water using Soxhlet apparatus and concentrated by rotary evaporator at 65° C. It was then transferred into a suitable container and freeze dried ready for the experiment. All preparations were performed at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin city, Edo State, Nigeria.

**Treatment of animals:** In the main research protocol, animals in group A received the aqueous extract of *P. amarus* at a single dose of 400mg/kg body weight daily for thirty days through the orogastric tube whilst animals of group B received a single dose of 800mg/kg body weight daily via the same route and the same period. Animals in group C received equal volume of distilled water, for the same period and through the same route of administration. Rats were euthanized by cervical dislocation on the thirty-one day of the experiment and the stomach and the duodenum were immediately dissected out and fixed in 10% formaldehyde- saline for routine histological techniques.

**Histological study:** Specimens were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax.

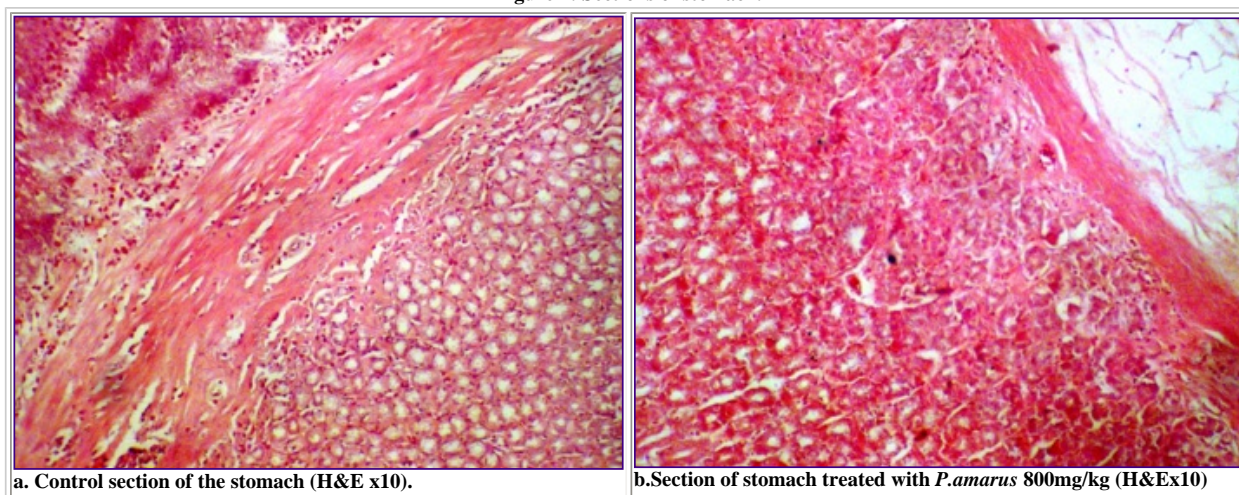
Serial sections of 7  $\mu\text{m}$  thick were obtained using a rotatory microtome. The deparaffinized sections were stained routinely with haematoxyline and eosin (H&E). Photomicrographs were obtained using research photographic microscope in the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin city, Edo State, Nigeria.

Selection criterion: It has been speculated that toxic effects could be independent of concentration<sup>16</sup>. In earlier report on kidneys, we registered no remarkable difference between the treated groups<sup>17</sup>. Sections were discretionally selected from groups [B] and [C] only for comparison.

## RESULTS

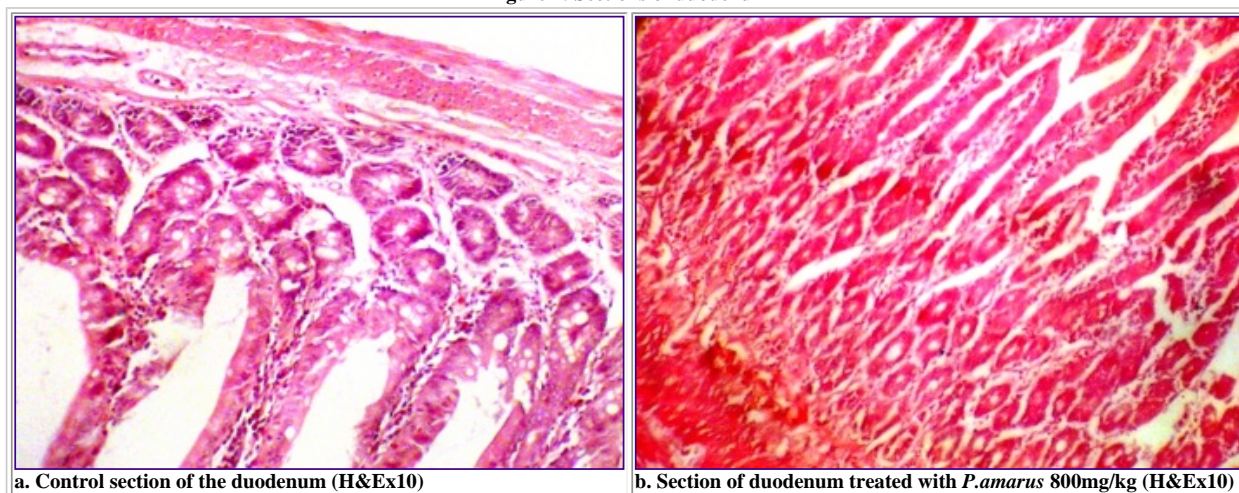
The photomicrograph of the stomach section from the control group showed normal histological features with the mucosa lined with simple columnar epithelial cells, lamina propria with some highly packed glandular secretory cells. The muscularis mucosa of the stomach was well noticed in the control section (Fig.1a). The sections of the stomach treated with *P. amarus* revealed some varying degree of distortion in the epithelial layer. There were obvious sign of proliferation, and atrophic changes in the treated stomach sections (Fig. 1b).

Figure 1: Sections of stomach.



The duodenum section from the control group [C] also evidenced normal histological features. There were indications of the presence of Brunner's gland and other cells lining the duodenum (Fig. 2a). The duodenum treated with *P. amarus* showed signs of erosion and inflammation, which are suggestive of ulcerations. The photomicrograph revealed some distortion and disruption in the epithelial lining, which may be suggestive of atrophic duodenitis (Fig. 2b)

Figure 2: Sections of duodenum



Limitations: The observations from this study have limitations. Gender differences among the rats within groups were not investigated. As explained in selection criteria, difference between groups A and B were not considered in this particular evaluation. Only the group B animals have been compared with control group C in this study.

## DISCUSSION

Since administration of *P. amarus* was the only differential treatment between treated and untreated groups, we guess that the registered toxic effects (Figures 1 and 2) may be attributed to the phytochemical content of *P. amarus*.

These results not only were congruent with a former report related with the toxic effects of red pepper administration on the cytoarchitecture of the stomach<sup>18</sup>, and with others found in the available literature<sup>12-13</sup>. In contrast, our findings differ from those of Lawson-Evi et al,<sup>19</sup> who reported no toxic effect or anatomical changes in digestive organs with *P. amarus*. However, it must be outlined that



these authors employed low doses of the plant.

Considering the known health benefits of the herbal agent<sup>8-11, 20-22</sup> it may be also guessed that the excessive and prolonged intake of *P. amarus* resulted in the aforesaid toxic effects on the epithelial lining. One of the major components of *P. amarus* is the antioxidant tannin, basically a phenolic compound, whose excessive and/or indiscriminate consumption can be toxic<sup>23</sup>. *P. amarus* also contains alkaloids and lots of other antioxidants<sup>5, 9</sup>, which has been administered in this study to apparently healthy animals. Thus, our observation is consistent with the notion that antioxidants may be associated with toxicities, particularly when arbitrarily consumed<sup>24</sup>.

Exceeding that antioxidants are essential for alleviation of oxidative stress, indiscriminate intake of alkaloids and antioxidant constituents of *P. amarus* may induce oxidative stress<sup>25-26</sup>. Our results are indicative that arbitrary chronic and/or excessive consumption of *P. amarus* may be harmful for animals.

In clinical practice, patients with peptic ulcer are advised to abstain from pepper. Taking into account the similar toxic effects produced on the cytoarchitecture of the stomach by red pepper administration<sup>18</sup>, and the results here reported, a rational basis is provided to hypothesize that ethnomedicinal practice involving the use of *P. amarus* may be contraindicated in peptic ulcer patients, especially if the herbal agent is required to be administered in high dosage. Summing up, it calls for caution and discretion as occurs with other medicines.

## CONCLUSION

This study presents histological evidence supporting that chronic and excessive administration of *P. amarus* has potential adverse effect on the microanatomy of the stomach and the duodenum. The hypothetical implication is that the function of digestion and absorption may be adversely affected by *P. amarus*. Last but not the least, we hypothesize the *P. amarus* may be contraindicated in stomach ulcer patients. Further analyses are recommended to verify this hypothesis.

## Acknowledgement

This work is part of Josiah Adjene's main research supported by the University of Benin. Some of the laboratory work for this particular report was done by Imuentinyan Abudu as part of his undergraduate study. Dr. Uba Nwose contributed the Translational Biomedical Science concepts. The authors gratefully acknowledge the support from various staff of the department of Anatomy of the University of Benin, Nigeria.

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Comment of the reviewer Daniel Al Kassam PhD. Biochemistry. Complejo Asistencial Universitario de Burgos. España.

En el presente trabajo Adjene et al. demuestran que *Phyllanthus amarus* produce daño en ratas. El paso siguiente sería identificar cual de estos componentes (phyllanthine, hypophyllanthine, alcaloides, quercetina, quercetrin, isoquercitrin, astragalín, amarín, geraniin, corilagin, rutina y 3-O-glucopyranoside) pueden ser los culpables del daño histológico.

Son necesarios estudios para identificar el o los agentes causales. Además, sería de utilidad valorar a partir de que dosis de cada compuesto aparece el daño.

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Comment of the reviewer Prof. Dr. Alberto Enrique D'Ottavio. Catedrático de Histología y Embriología. Facultad de Ciencias Médicas. Universidad Nacional de Rosario. Rosario. Argentina

Este trabajo descriptivo histológico de los efectos tóxicos de *Phyllanthus amarus*, administrado en altas dosis, sobre estómago y duodeno de ratas Wistar aborda un tema de interés ya que incursiona en la práctica etnomedicinal, de innegable uso en determinados ámbitos.

Sus resultados, si bien no son directamente extrapolables al ser humano y se han obtenido mediante técnicas histológicas corrientes, no resultan descartables pues sirven, por un lado, para advertir indiciariamente sobre los riesgos del uso crónico, indiscriminado y nada sistemático del *P. amarus* (de similares efectos a la pimienta roja en el estómago), y, por otra parte, para alentar profundizaciones investigativas sobre este menester.

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Received September 09, 2010. Received reviewed January 12, 2011  
Published: April 16, 2011