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Aqueous Extract of *Khaya Senegalensis* Erodes the Luminal Layer of the Duodenum in Wistar Rats

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ABSTRACT

Introduction: *Khaya Senegalensis* Desr. is one of the plants commonly used in Nigeria and several West African countries, as a medicinal plant. One way of assessing the effect of medicinal plants is to determine its effect on the histology of various organs. Changes from the normal histo-architecture would denote an adverse effect of the extract on the administered organ. The present study was undertaken to determine the effect of the aqueous *Khaya Senegalensis* stem bark extract on the histology of the duodenum and the pancreas.

Methodology: four groups of albino rats were administered different dosages of the extract orally and after 28 days, the rats were sacrificed. Micrographs of the duodenum and pancreas were studied for observations. Morphometric analysis was also carried out to determine the thickness of the epithelial lining in all groups. Measurements were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test using GraphPad Prism.

Results: the extract distorted the histological arrangement of the duodenum at a concentration above 50mg/kg by causing an erosion in the layers starting from the epithelial lining. The presence of several inflammatory cells in the lamina propria of rats that received the extract at a dosage of 100mg/kg is indicative that the erosion of the epithelial lining elicited an inflammatory reaction. There was little histological change observed in the histology of the pancreas

Conclusion: the result of this study indicates that the aqueous stem bark extract of *Khaya senegalensis* may affect the cellular integrity of small intestine in a dose-dependant manner.

Keywords: *Khaya senegalensis*; Lamina propria; Epithelium; Duodenum; Intestine.

Introduction

Plants that possess therapeutic properties or exert beneficial pharmacological effects on the body are generally designated as "medicinal plants"[1,2]. The practice of using medicinal plants as medicinal remedy is gaining acceptance in developed and developing nations as researchers are beginning to harness the potentials in healthcare delivery[3]. In addition, medicinal plants are affordable and available in many developing regions as an alternative source of medical aid and therapy.

Khaya Senegalensis Desr. is one of the plants commonly used in Nigeria and several West African countries, as a medicinal plant[4] . The bark extract has been used for treating jaundice, dermatoses, hookworm infection and malaria[5]. The seeds and leaves are used to treat fever and headache; and the roots are used to treat mental illness, syphilis and leprosy[5,6]. *Khaya Senegalensis* extracts have been reported to exhibit antiinflammatory effects as well as anti-bacterial, antihelmintic, antitumor, antioxidant and antiplasmodial activities. The stem bark extract and the chemical constituent profile have been the subject of extensive phytochemical and pharmacological investigations since the 1960s[7].

The liver of mammals serve the important role of detoxification of foreign compounds and regulation of blood glucose level, among others functions, the kidneys serve as organs that filter blood and is exposed to metabolites in the body, these organs are investigated primarily to determine the effect of metabolites ingested. However, the gastrointestinal tract directly receives the extract when it is administered through the oral route and could be subject to histological changes as a result of exposure to the chemical constituent present in the extract. Just as the liver receives blood draining the gastrointestinal tract and becomes exposed to chemicals, poisons and toxins absorbed from the gut, the gastrointestinal tract also receives the aqueous extract as directly administered. Many research studies have been conducted to determine the effect of *Khaya Senegalensis* extract in many forms to several organs including the biochemical and heamatological assays to determine the effect of the extract on kidney and liver function but the authors discovered a dearth of information on the effect of the

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extract on the gastrointestinal tract. The current study was carried out to determine if the aqueous extract of *Khaya Senegalensis* has any effect on the histology of the gastrointestinal tract.

Methodology

Plant Collection, Authentication and Extraction

Khaya Senegalensis bark was obtained from the University of Maiduguri, Borno State, Nigeria. The plant was authenticated by a Botanist in the Department of Biological Sciences, Faculty of Medical Sciences, University of Maiduguri.

Khaya Senegalensis stem bark was air dried for a period of 14 days after which it was pounded and sieved to obtain the powdered form. The powdered stem bark was measured and found to weigh 647.3g. A solution of the powdered stem bark in distilled water was made and placed in an electrical oven for a period of 3 days for dehydration after which the dehydrated stem bark was dissolved in water to obtain the aqueous extract.

Animal Husbandry

Twenty-four (24) albino wistar rats were purchased from the Department of Veterinary Medicine, Ahmadu Bello University, Kaduna state. The animals were kept in the animal house of the Department of Human Anatomy, for one week to acclimatize before the experimental process began. They were housed in well ventilated plastic cages at room temperature and hygienic conditions under natural light (13 hours) and dark (11 hours) schedule and were fed on standard rat pellet and water *ad libitum*.

Experimental Design and Treatment Protocol

The twenty-four (24) rats were assigned into four groups with six (6) animals in each group using the LD 50 of 5000mg/kg as determined by (Kolawole *et al*., 2011). The extract was administered to treatment groups daily at the same time period for 28 days (Table 1).

Table 1. Treatment protocol for the experimental groups.

Groups	Treatment Protocol
Group A	50mg/kg water
Group B	50mg/kg of Khaya Senegalensis stem bark extract
Group C	100mg/kg of Khaya Senegalensis stem bark extract
Group D	200mg/kg of Khaya Senegalensis stem bark extract

Animal Sacrifice

The animals were humanely sacrificed after four weeks using ketamine anesthesia which was administered to the left thigh of the animals. The organs of interest were dissected from an incision made on the surface of the anterior abdominal wall to access the abdominal cavity. The small intestine and pancreas were dissected out, fixed in 10% formalin in preparation of routine histological preparation.

Tissue Processing and Observation

The intestine and pancreas were fixed in 10% formalin (Balaji Formalin Private Limited, India), and then, the tissue was washed under running water before it was dehydrated using alcohol (Sigma-Aldrich, USA). This process was carried out by immersion of the specimen in a graded series of ethanol (alcohol) solutions of increasing concentration until absolute alcohol was used. Xylene (Veckridge Chemicals, New Jersey) was used to clear the alcohol found in the tissue and prepare it for the next stage of tissue preparation. The tissue was infiltrated with wax and embedded to form a tissue block which was clamped into a microtome for sectioning using a microtome (Leica RM2125 Rotary Microtome) and stained with Hematoxylin and Eosin (Abbey Colour, Philadelphia). The embedding process was reversed to dewax the tissue and allow dyes to penetrate the sections by running them through xylene to clear and alcohol to rehydrate. The tissue was stained with Hematoxylin and Eosin (Abbey Colour, Philadelphia). The sections were then photographed using an Amscope light microscope (MBJX-ISCOPE, Los Angeles) with a digital camera (M500, X 64, version 3.7) under X40 and X200 magnifications. Images of the histological sections were photographed using 10X objective lens and these images are presented as results.

Morphological Dtudies

The thickness of the layers of the duodenum was measured using image J application (National Institutes of Health, USA, Version 1.35k) tracing tool to trace a line from the luminal border of the simple columnar epithelial cell to the adventitial layer. These measurements were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test using GraphPad Prism (version 8.0.2). The results were represented as a bar graph with the X axis representing the groups and the Y axis showing the thickness of the duodenum in □m.

Compliance with Ethical Standard

The experimental procedures were conducted in accordance with the University of Maiduguri Research and Ethical Committee guidelines, the ARRIVE guidelines (reporting of in vivo experiment), and the National Institutes of Health (NIH) guide for the CARE and use of laboratory animals (NIH Publications No. 8023, revised 1978). The research was also conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Results

Morphometric Analysis of the Duodenum

The measurement of the thickness of the duodenum is presented in figure 1. The thickness was highest in group A with a length of 122□m. The length decreased along the treatment groups with the animals who had the highest dose in group D having the shortest distance. The length was statistically significant ($p<0.05$) in groups C and D when compared to the control group. The decrease in length was not statistically significant in group B (Figure 1)

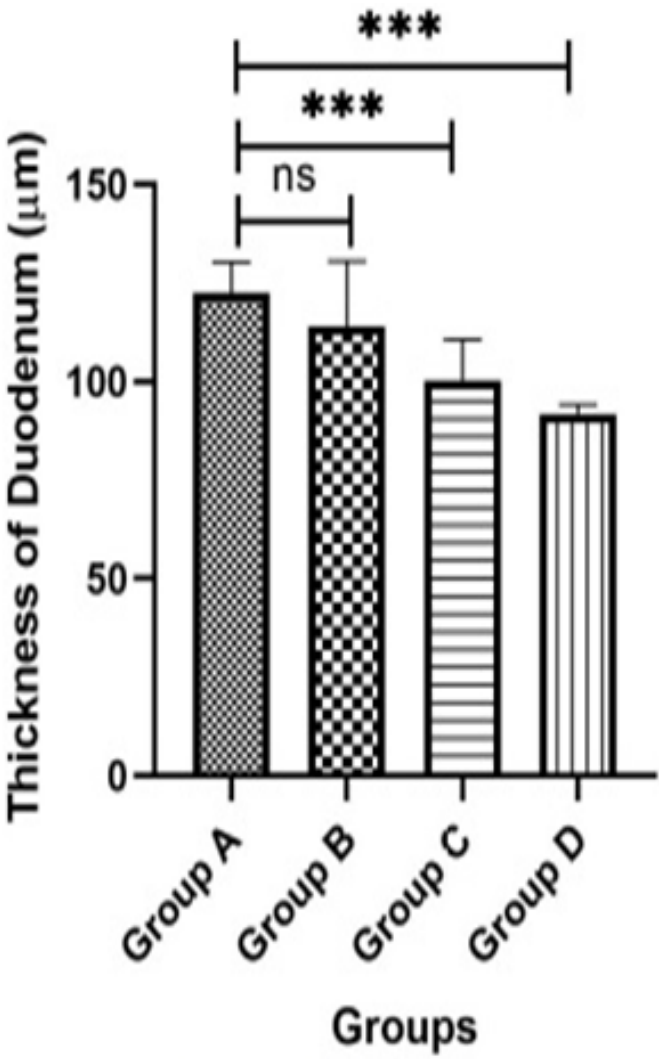

Figure 1. Showing the thickness of the duodenum in all groups.

Histological Observations in the Duodenum and Pancreas

The micrograph representing the histology of the duodenum is presented in Figures 2A-D and 3A-D. The control group is presented in figures 2A and figure 3A showing the layers of the gastrointestinal tract in animals that did not ingest the extract. The epithelial lining was thrown into finger-like folds referred to as intestinal villi. These extended into the intestinal lumen and consisted of simple columnar epithelial cells which were notable by their eosinophilic cytoplasm and basal nuclei. Interspersed between these cells were goblet cells which are mucous secreting in function. At the base of the intestinal villi were crypts of Lieberkuhn which release intestinal secretion received from intestinal glands into the lumen. The connective tissue layer below the epithelium (lamina propria) contained cells hose dark staining nuclei were easily identified. Muscularis mucosa was located at the base of the crypts and below that was the submucosal layer which was another connective tissue layer with blood vessels and cellular constituents present. The muscular layer which was oriented in two layers and had a plexus of nerves sandwiched in between the two layers. The adventitial layer was sparse and few cells were found in this region.

Micrographs representing the duodenum of rats in group B were similar to the control group with an increased number of goblet cells compared to group A (figures 2B and 3B). More crypts were are found at the base of the epithelial cells and these also had more goblet cells. The muscular layer was thinner than in the muscular layer found in the control group. The rats in group C showed erosion on the intestinal villi with more goblet cells found here than in the other groups (figure 3C). The muscular layer was thicker than in group B (figure 2C). The duodenum in rats in group D is represented in figures 2D and 3D. The intestinal villi was eroded with epithelial cells and goblet cells mostly lost (figure 3D). The intestinal crypts were present and also numerous in number and had wider lumen compared to the other groups. Muscularis mucosa was discontinuous and several blood vessels were found in the submucosa and muscular lining was also thick in this group (Figure 3D). Inflammatory cells were found in the lamina propria of cells in group C. They were not so prominent in group D as the epithelium was eroded in this group and many cells in the lamina propria were indistinguishable.

The micrograph showing the pancreas is represented in figures 4A-D. The control group shows both endocrine and exocrine pancreas. The endocrine pancreas reveals the islets of Langerhans consisting of a clump of secretory cells which are pale-staining and surrounded by a thin layer of connective tissue (Figure 4A). The exocrine pancreas consisted of pyramidal cells which formed pancreatic acini. The acini were arranged into alveolar subunits of the gland. The luminal content was stained with eosinophilic material and a few intralobular ducts were seen in the glandular parenchyma. The other groups that were administered with the extract had larger pancreatic acinar cells compared to the control group. Fewer islets were found in the pancreatic parenchyma when compared to the control group (Figures 4C and D).

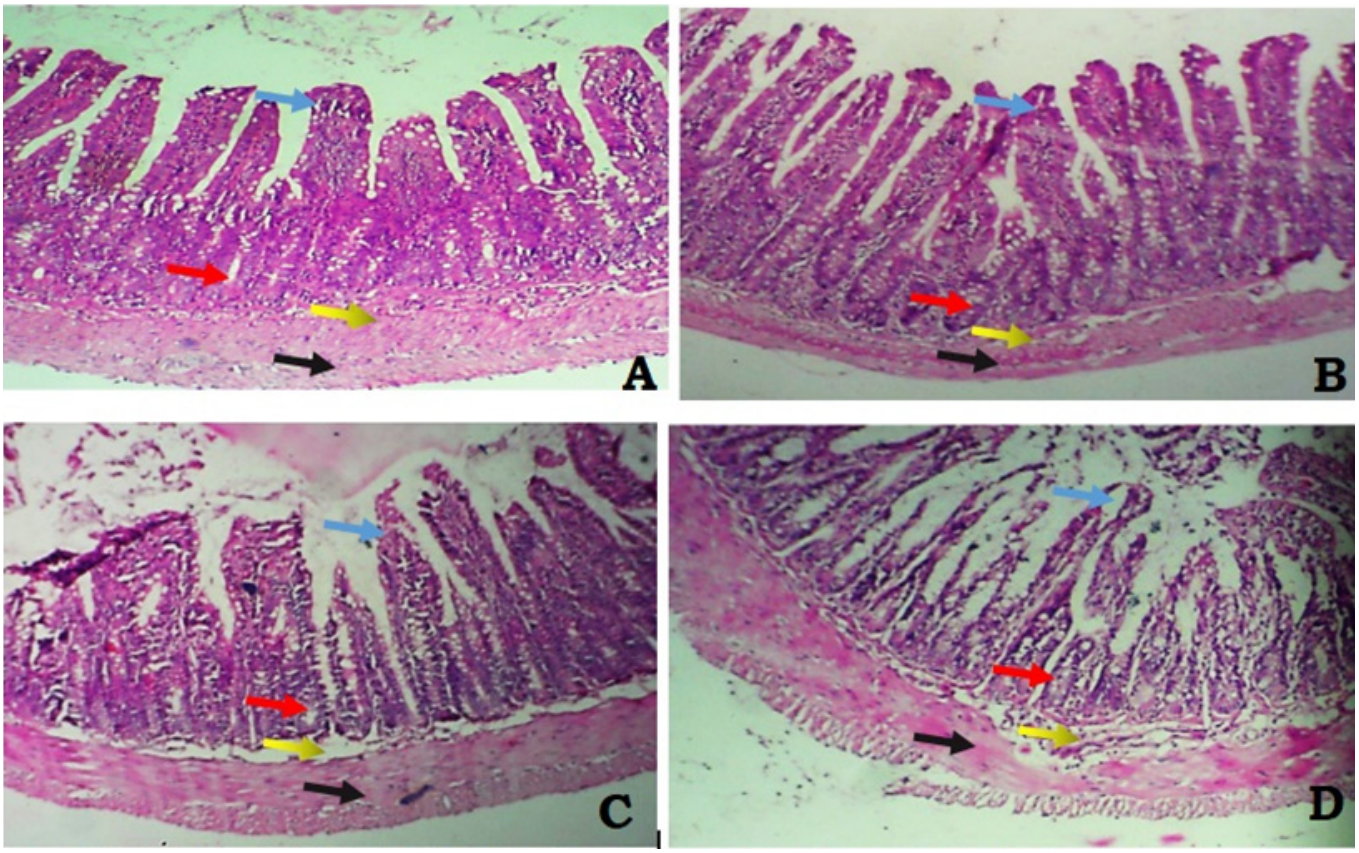

Figure 2. A-D showing the micrograph of the duodenum showing the entire layers. The blue arrows represent intestinal villi which projectedinto the intestinal lumen. The villi were eroded in group D. The crypts of Lieberkuhn (red arrow) were found at the base of the villi and lay above the submucosal layer (yellow arrow). The muscular layer (black arrow) was thickest in group D. H&E x 40.

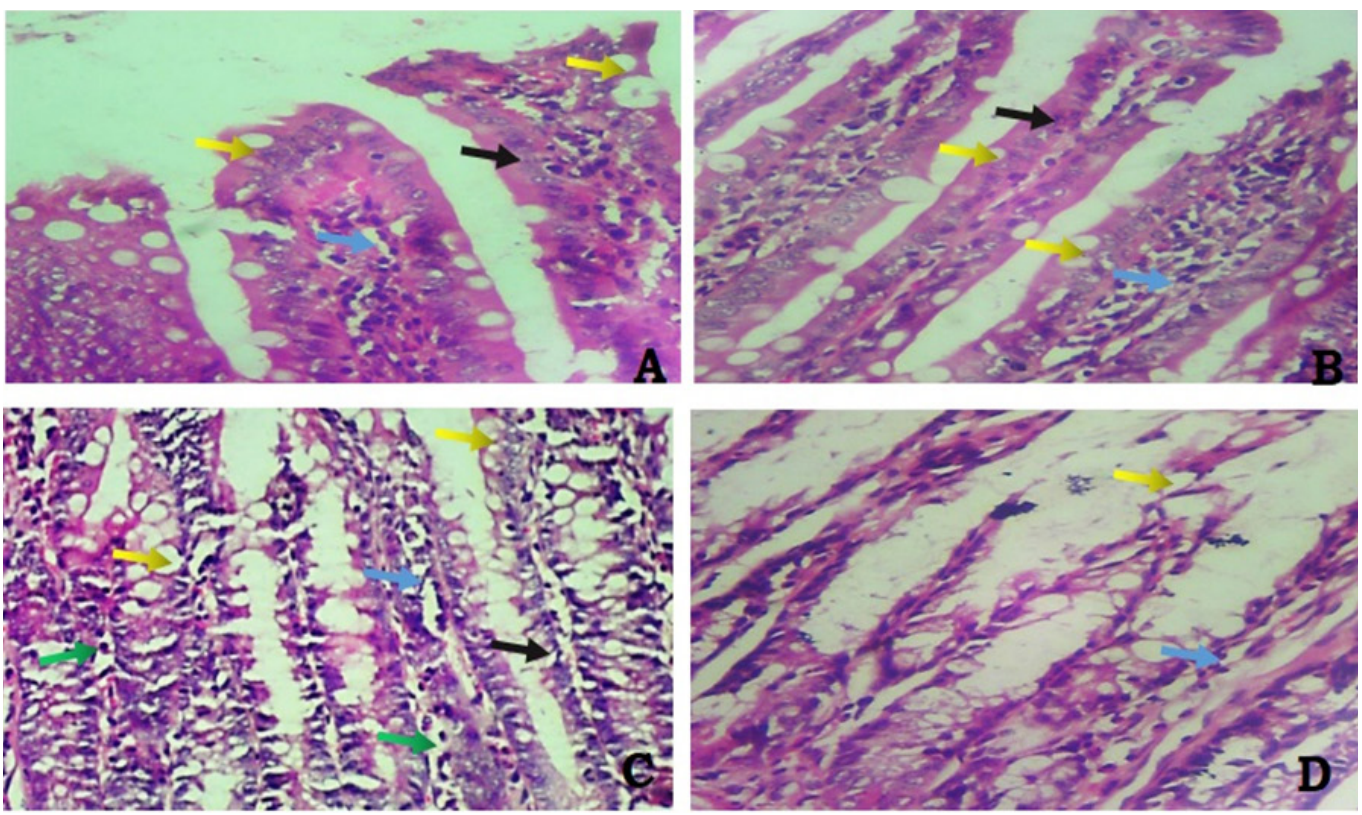

Figure 3. A-D showing the intestinal villi in all groups at higher magnification. The simple columnar epithelial cells (black arrow) lined the villi and had goblet cells (yellow arrow) interspersed between the. These were more numerous in group C and fewst in number in group D. The lamina propria (blue arrow) was directy exposed to the luminal surface in group D. Inflammatory cells (green arrow) were located in the lamina propria in group C. H and E x200.

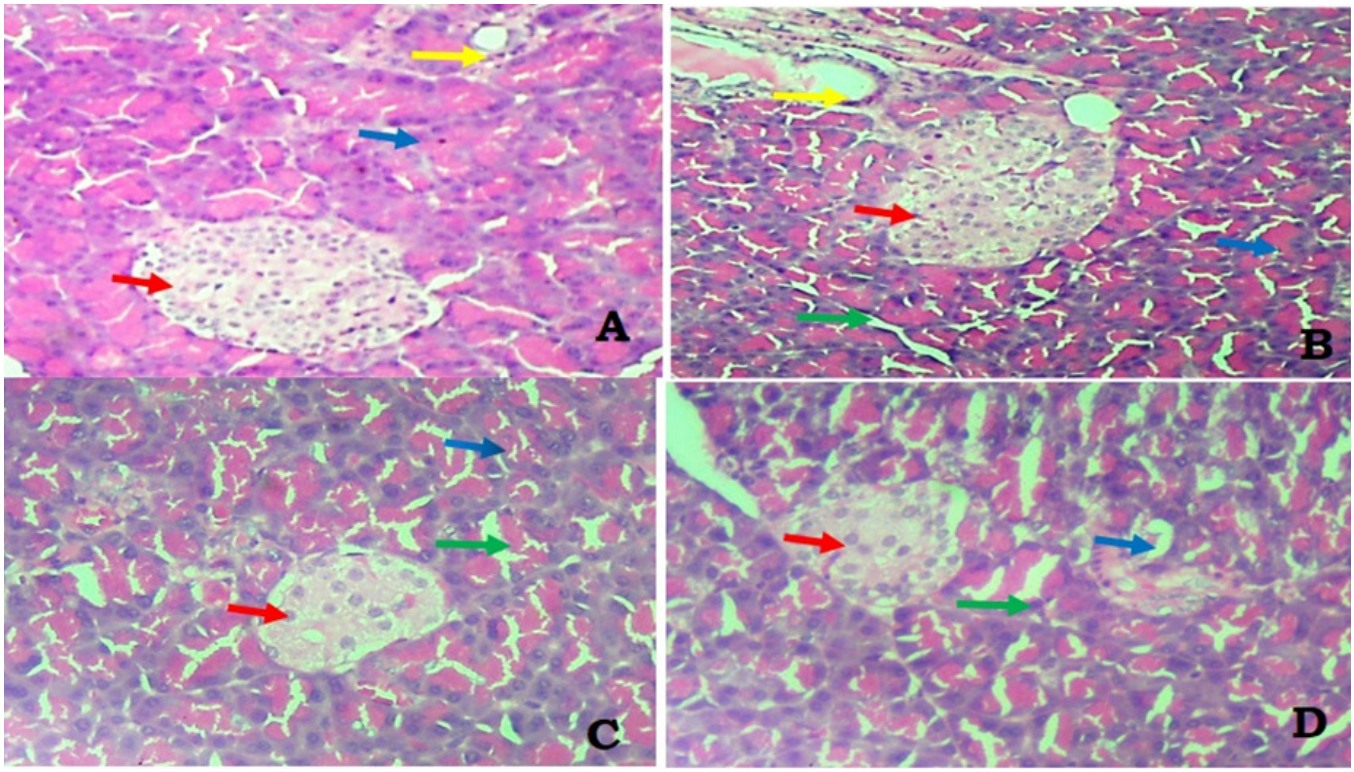

Figure 4. A-D representing the pancreas of the rats in all groups with red arrows pointed on islets of Langerhans wich were smallerin size in the groups that received higher concent the extact compared to the control and low dose groups. The exocrine pancreas also had acinar cells (blue arrow). Yellow arrow is on interiobular duct. H&E x100.

Discussion

Alternative medicine comprises of medical knowledge system that is developed over generations within various societies before the era of modern medicine[8]. The use of medicinal plants as medical therapy involves the use of natural things (mostly plants) to treat various diseases. This practice has become widespread and accepted in many societies around the world as a result of the effectiveness, low cost and the availability of these herbal medicines [7,8,9]. The widespread and prolonged use of herbal medicines do not guarantee their efficacy and safety[10]. Therefore, there is a need for detailed scientific analyses and adequate information on the toxicity and effects of commonly used herbal medication[8,11]. One way of assessing the effect of medicinal plants is to determine its effect on the histology of various organs. Changes from the normal histo-architecture would denote an adverse effect of the extract on the administered organ. The present study was undertaken to determine the effect of the aqueous *Khaya Senegalensis* stem bark extract on the histology of the duodenum and the pancreas.

The extract was found to distort the histological arrangement of the duodenum at a concentration above 50mg/kg by causing an erosion in the layers starting from the epithelial lining. The presence of several inflammatory cells in the lamina propria of rats that received the extract at a dosage of 100mg/kg is indicative that the erosion of the epithelial lining elicited an inflammatory reaction. This information is consistent with studies that observed that *Khaya Senegalensis* stem bark extract at a concentration of 500mg/kg caused inflammatory cells to migrate towards the central vein as well as collapse of hepatic sinusoids of rats treated with the extract [8]. Another study showed that the liver of rats treated with the extract from a concentration of 400mg/kg showed cellular degeneration and necrosis along with bile duct hyperplasia and fibrosis with lymphocytic infiltration of the hepatocyte providing supportive evidence indicating functional derangement[7]. In that study, the histological architecture of the kidney and that of the heart were preserved. Further studies conducted indicated that prolonged administration of *Khaya Senegalensis* bark extract at a concentration of 50mg/kg of body weight mildly affected some biochemical parameters, however, no active damage was reported as penetrating beyond the cytoplasm[1].

It was demonstrated that ethanolic extract of dried leaves of *Khaya Senegalensis* had antispasmodic and spasmolytic effect on the muscular layer of guinea pig ileum[12]. Another study evaluated the in-vivo the effects of stem bark aqueous and ethanolic extracts on the digestive transit and faeces wetness in albino rats and observed a dose-dependent decrease of the diarrhoea without significant decrease in bowel transit at a concentration of 100-300mg/kg. The histology of the ileum however was not determined in that study[13].

The extract caused very little histological changes to the exocrine and endocrine pancreas in the current

study although the islets found in the groups that received higher concentration of the extract appeared smaller in size and the extract may exert a negative effect on the proliferation of exocrine cells. This however has to be subjected to further study as there us a dearth of information on the effect of the extract on the histology and function of the pancreas.

Conclusion

In the present study, *Khaya Senegalensis* leaf extract was found to cause changes in the cellular integrity of the cells of the duodenum, causing an erosion to the epithelial lining and infiltration of inflammatory cells into the lamina propria at a concentration greater than 100mg/kg. From the result of the present study, the prolonged usage of extract should proceed with caution at a dosage greater than that indicated. Further studies are required to understand the constituent responsible for the disruption of cellular integrity and the mechanism involved.

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