

JOPAT Vol 24(1), 2054 – 2062. Jan – June, 2025 Edition.

ISSN2636 – 5448.

<https://dx.doi.org/10.4314/jopat.v24i1.22>

Histological Evaluation of Ethanol Leaf Extract of *Syzygium aromaticum* on Stomach and Colon of Wistar Rats.

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ABSTRACT:

The use of food additives or herbs has consistently received attention from researchers globally. One of these substances, *Syzygium aromaticum*, is used for medicinal purposes and food spices. This work evaluated the histological effect of ethanol leaf extract of *Syzygium aromaticum* on the stomach and large intestine of adult Wistar rats. Twelve-four (24) adult Wistar rats with average weight of 200 g were randomly divided into four groups of six rats each. Group A served as the control and received 1 ml of water, Group B, C and D received 200, 400 and 800 mg/kg body weight of ethanol leaf extract of *Syzygium aromaticum* respectively. All administrations were done through oral route for 21 days. After sacrifice, the stomach and colonic tissues were harvested, fixed in 10% neutral buffered formalin and processed for haematoxylin and eosin staining. Histological findings in stomach showed normal mucosal crypts, activation of lamina propria lymphocytes, mural active vascular congestion and dilatation in the 200 mg/kg body weight treated group; heavy mural infiltrates of eosinophils, mural vasodilatation and congestion in the 400 mg/kg body weight treated group; heavy mural infiltrates of inflammatory cells, mural vasodilatation and congestion in the 800 mg/kg body weight treated group. Histological findings in colon at all doses revealed normal colonic architecture of mucosa crypts, increased goblet cells, muscularis propria and activation of lamina propria lymphocytes. Conclusively, *Syzygium aromaticum* could be considered toxic on the stomach at a dose exceeding 200 mg/kg body weight, but with no significant effect on the colon. Thus its culinary and medicinal consumption should be monitored.

Key Words: *Syzygium aromaticum*; inflammatory cells; mural vasodilatation; stomach; colon

INTRODUCTION

The network of organs that aid the digestion and absorption of food particles is the digestive system, which includes the gastrointestinal tract and biliary system [1]. Stomach and colon are important parts of the gastrointestinal tract, and thus, any ingestion of substance through this tract must be regulated [2]. The stomach is a hollow and muscular organ present in humans and other animals which aids digestion of food

after chewing by enzymatic and acidic nature of its lining [3]. The stomach is found between the oesophagus and small intestine. The passage of food through the stomach is guided by sphincter through the duodenum and the rest of the intestine by peristaltic movement [4]. On the other hand, the colon is a tube-like organ that connects to the small intestine at one end and the anus at the other [5].

It is the longest part of the large intestine. The colon acts on partially digested food by removing water and electrolytes and nutrients [6]. The left over solid waste passes through the colon to the rectum where it is stored before removal through the anus [7].

Syzygium aromaticum (clove) is a dried flower bud belonging to the Myrtaceae family that is indigenous to the Maluku islands in Indonesia but has recently been farmed in different places worldwide [8]. It is a median size tree (8-12 m), frequently cultivated in coastal areas at maximum altitudes of 200 m above the sea level. *Syzygium aromaticum* is commercially used for many medicinal purposes and in the perfume industry, and is considered one of the spices that can be potentially used as preservatives in many foods, especially in meat processing, to replace chemical preservatives due to its antioxidant and antimicrobial properties [9]. *Syzygium aromaticum* in particular has attracted attention due to its potent antioxidant and antimicrobial activities standing out among the other spices [10]. In West Africa, the Yorubas use *Syzygium aromaticum* infused in water as a treatment for stomach upsets, vomiting and diarrhoea. The infusion is called “Ogun Jedi-jedi” [11]. Studies have supported the use of *Syzygium aromaticum* and *Syzygium aromaticum* oil for dental pain, and to a lesser extent for fever reduction, as a mosquito repellent and to prevent premature ejaculation [12]. *Syzygium aromaticum* may reduce blood sugar levels [13]. The effective role of *Syzygium aromaticum* in the inhibition of different degenerative diseases is attributed to the presence of various chemical constituents such as hydroxibenzoic acids, flavonoids, hydroxyphenyl propens, hydroxycinnamic acids, and eugenol—which is the major bioactive molecule [14]. *Syzygium aromaticum* essential oil is traditionally used in the treatment of burns and wounds, and as a pain reliever in dental care as well as treating tooth infections and toothache [15]. In addition to that, its use has been documented in various industrial applications and is used extensively in perfumes and soaps [16]. *Syzygium aromaticum* is used in Indian and Chinese traditional medicine as a warming and

stimulating agent [17]. In tropical Asia, *Syzygium aromaticum* has been documented to relieve different microorganisms as scabies, cholera, malaria, and tuberculosis [18]. Traditionally, *Syzygium aromaticum* has been used for centuries in the treatment of vomiting; flatulence; nausea; liver, bowel and stomach disorders [19, 20]. It is a well-known and significant herbal remedy because of its broad pharmacological efficacy [21, 22]. Recent studies have examined the in vivo increase in the lipid peroxidation and blood sugar in diabetic rats and reestablished the levels of the antioxidant enzyme after nutrition supplement with *Syzygium aromaticum* [23]. The aim of this work evaluated the histological effect of ethanol leaf extract of *Syzygium aromaticum* on the stomach and large intestine of adult Wistar rats

MATERIALS AND METHOD

Collection, Identification and Preparation of Plant Material:

Fresh *Syzygium aromaticum* leaves were harvested in Ekpoma community in Edo State. It was identified and authenticated at the Plant Science and Technology Department of Ambrose Alli University, Ekpoma, Edo State. The leaves were thoroughly washed with tap water, air-dried and then pulverized to powdered form. 200g of the powdered form was soaked in 500ml of 70% ethanol for 72 hours. The crude ethanol extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was freeze-dried, using the method of Kumar [24] and refrigerated at -4 Celsius until use.

Experimental Animals:

Twelve-four [24] Wistar rats weighing 115-292 g were procured and housed in the Animal House, Department of Anatomy, Ambrose Alli University, Ekpoma, Edo State. Water and food were provided *ad libitum*. They were exposed to controlled environmental temperature ($28 \pm 2^\circ\text{C}$), relative humidity ($50 \pm 5\%$) and 12 hour light-dark cycle.

Dosage selection:

The plant extract (10g) was dissolved in 100 ml of water and was administered orally to Wistar

rats at the doses of 200, 400 and 800 mg/kg body weight after LD₅₀ (25) was done.

Animal Groupings:

The animals were randomly divided into four groups of six animals each.

Group A: (Control) received only 1ml of distilled water daily for a period of 21 days.

Group B: received 200 mg/kg body weight ethanol leaf extract of *Syzygium aromaticum* orally for a period of 21 days.

Group C: received 400 mg/kg body weight ethanol leaf extract of *Syzygium aromaticum* orally for a period of 21 days.

Group D: received 800 mg/kg body weight ethanol leaf extract of *Syzygium aromaticum* orally for a period of 21 days.

All the animals were sacrificed 24 hours after the last day of administration under chloroform anesthesia. Abdomino-thoracic incision was made on the rat to expose the abdominal viscera. Thereafter, the stomach and colonic tissues were harvested and immediately fixed in 10% formol saline in a universal bottle for histological analysis.

Histological analysis:

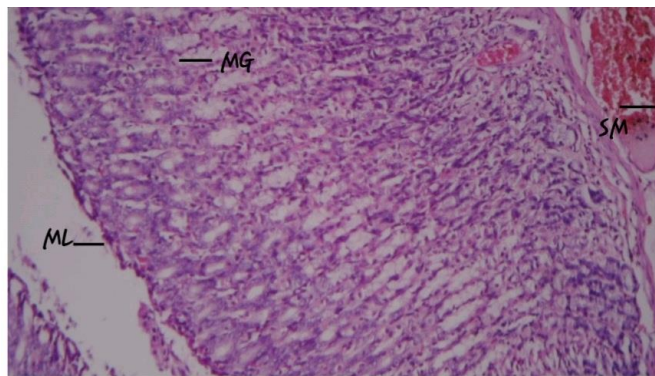


Figure 1. Photomicrograph of stomach histology of Group A (control) showing normal architecture: mucosal lining indented by pits (ML), mucosal glands (MG), submucosa (SM); H&E 100x

The formol-saline fixed stomach and colonic tissues were dehydrated in ethanol (70 to 100 %), cleared in xylene and embedded in paraffin. All tissue sections were examined under a light microscope after staining with hematoxylin and eosin [26].

RESULTS

Histological findings in stomach (Figure 1-4) showed normal mucosal crypts, activation of lamina propria lymphocytes, mural active vascular congestion and dilatation in the 200 mg/kg body weight treated group; heavy mural infiltrates of eosinophils, mural vasodilatation and congestion in the 400 mg/kg body weight treated group; heavy mural infiltrates of inflammatory cells, mural vasodilatation and congestion in the 800 mg/kg body weight treated group. Histological findings in the colon (Figure 5-8) at all doses revealed normal colonic architecture of mucosa crypts, increased goblet cells, muscularis propria and activation of lamina propria lymphocytes. Histological findings in colon at all doses revealed normal colonic architecture of mucosa crypts, increased goblet cells, muscularis propria and activation of lamina propria lymphocytes.

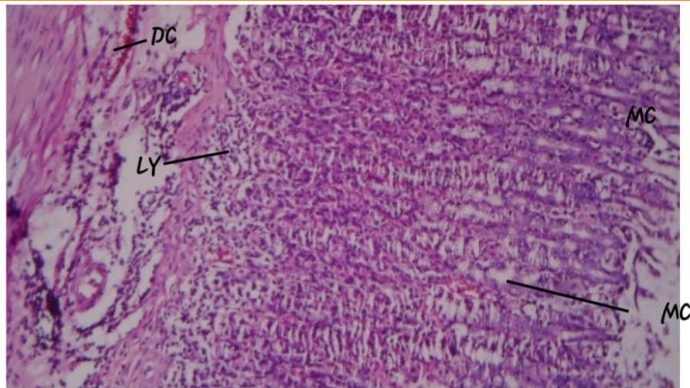


Figure 2. Photomicrograph of stomach histology of Group B (200 mg/kg between treated group) showing normal mucosal crypts (MC), activation of lamina propria lymphocytes (LY), mural active vascular congestion and dilatation (DC): H&E 100x

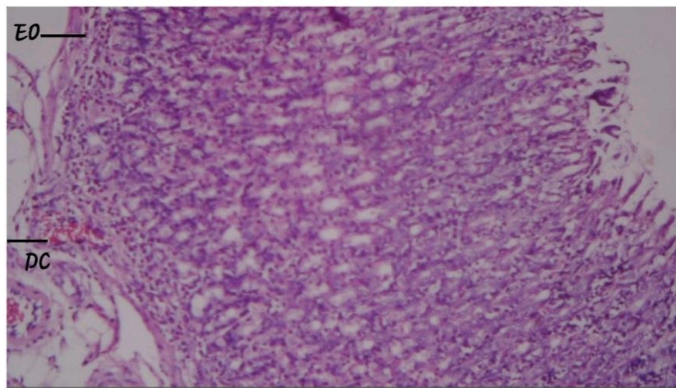


Figure 3. Photomicrograph of the stomach histology of Group C (400 mg/kg between treated group) showing heavy mural infiltrates of eosinophils (EO), mural vasodilatation and congestion (DC): H&E 100x

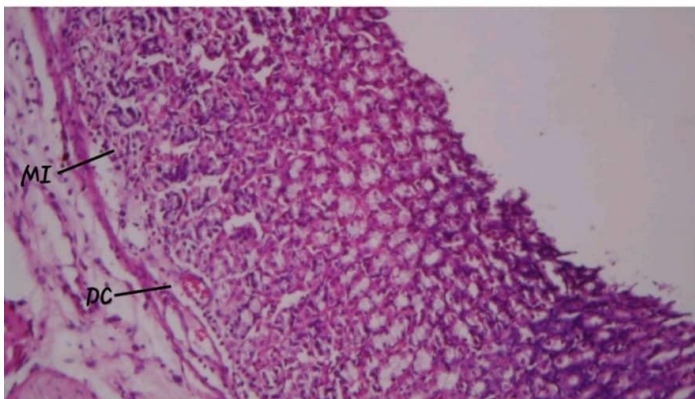


Figure 4. Photomicrograph of stomach of Group D (800 mg/kg between treated group) showing heavy mural infiltrates of inflammatory cells (MI), mural vasodilatation and congestion (DC): H&E 100x

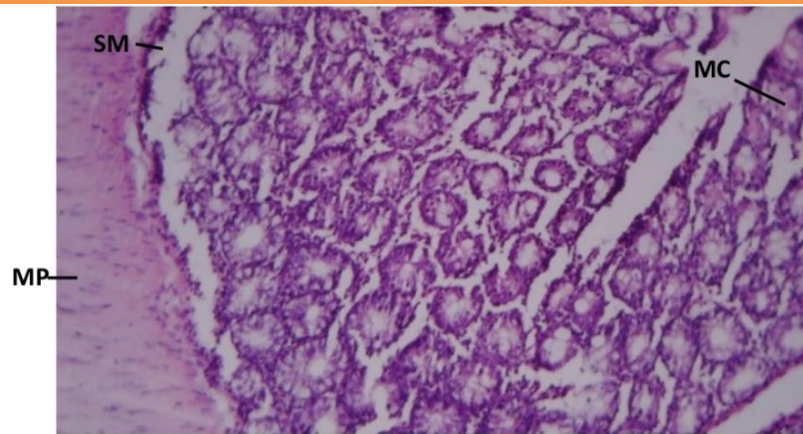


Figure 5. Photomicrograph of colon of Group A (control) showing mucosal crypts (MC), submucosa (SM), muscularis propria (MP), all normal: H&E 100x

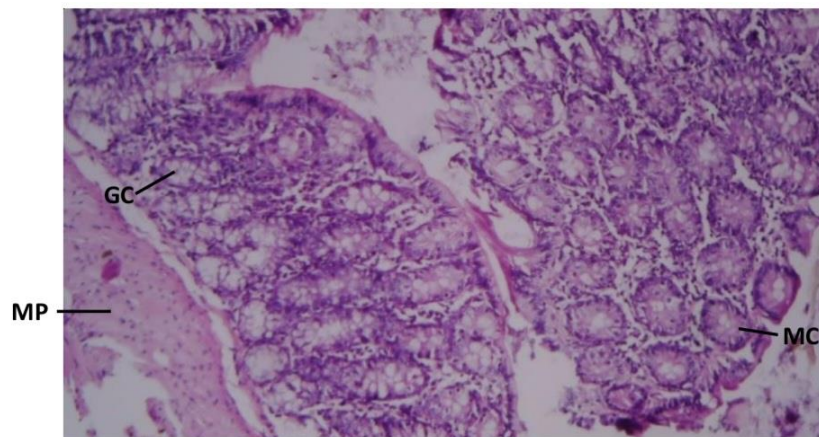


Figure 6. Photomicrograph of colon in Group B (200 mg/kg between treated group) showing mucosal crypts (MC), with increased goblet cells (GC), muscularis propria (MP), all normal: H&E 100x

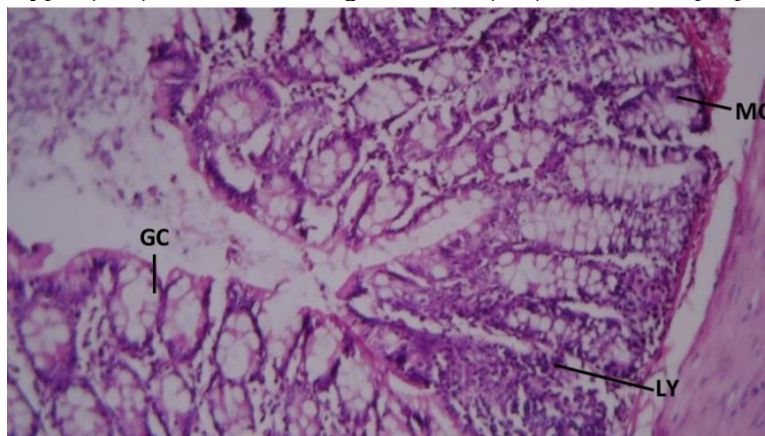


Figure 7. Photomicrograph of colon in Group C (400 mg/kg between treated group) showing mucosal crypts (MC), with increased goblet cells (GC), activated lamina propria lymphocytes (LY), all normal: H&E 100x

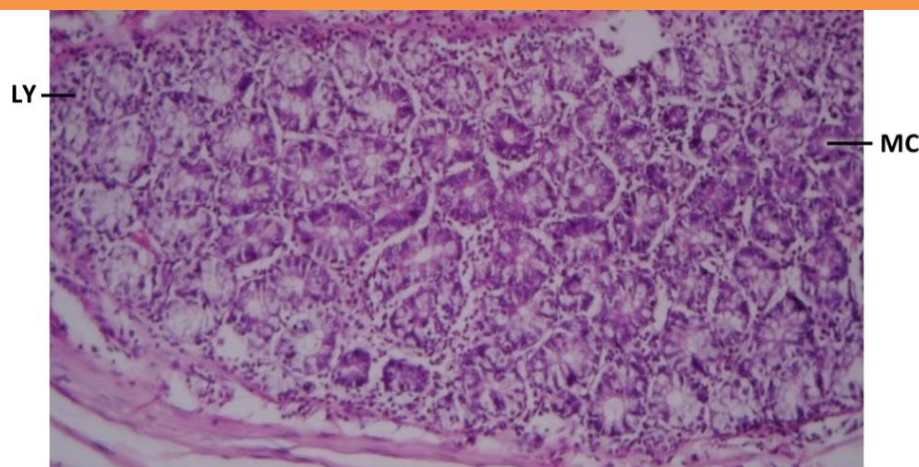


Figure 8. Photomicrograph of colon in Group D (800 mg/kg between treated group) showing mucosal crypts (MC), with activation lamina propria lymphocytes (LY), all normal: H&E 100x

DISCUSSION

The histological findings on the stomach showed that normal mucosal crypts, activation of lamina propria lymphocytes, mural active vascular congestion and dilatation were seen in the 200mg/kg treated group, heavy mural infiltrates of eosinophils, mural vasodilatation and congestion in the 400mg/kg treated group and heavy mural infiltrates of inflammatory cells, mural vasodilatation and congestion in the 800mg/kg treated group. The presence of infiltrates, mural vascular congestion and inflammation may indicate potential toxicological effects of the *Syzygium aromaticum* extract at the administered doses [27]. Certain compounds within the extract might lead to adverse reactions in stomach tissues. The presence of eosinophils could be as a result of the immune system's response to a foreign substance in the stomach. This finding correlates with Birchenough [28] who reported that immune system is of fundamental importance to gastrointestinal immunology. It is important to note that individual responses to any substance can vary, and in some cases, people may experience adverse reactions to certain herbs or spices, including cloves [29]. Allergic reactions or sensitivities to cloves could potentially lead to inflammation in some individuals, but such cases are relatively rare [29].

The histological findings on the colon showed no significant changes or damage to the tissue.

The results showed that the colon sections from all groups, including the control and treated groups, exhibited normal cytoarchitectures, including mucosal crypts, submucosa, muscularis propria, and lamina propria lymphocytes. The increase in goblet cells observed in the treated groups (200 mg/kg and 400 mg/kg) may indicate an adaptive response to the extract, potentially enhancing mucus production and protecting the intestinal epithelium. The activation of lamina propria lymphocytes seen in the 400 mg/kg and 800 mg/kg groups may suggest an immune response to the extract, which could contribute to the maintenance of intestinal homeostasis. These findings are consistent with previous studies that have investigated the effects of *Syzygium aromaticum* ethanol leaf extract on the gastrointestinal tract. For example, a study by Alqasoumi [30] found that *Syzygium aromaticum* ethanol leaf extract did not cause any significant histological changes in the stomach and intestines of rats. Similarly, a study by Kumar [31] and Allan [32] found that *Syzygium aromaticum* ethanol leaf extract exhibited no toxic effects on the colon and rectum of rats. Additionally, a study by Syed [33] found that *Syzygium aromaticum* ethanol leaf extract was safe for consumption and did not cause any significant adverse effects on the intestines..

CONCLUSION

Syzygium aromaticum ethanol leaf extract could be considered toxic at dose exceeding 200

mg/kg of body weight on the stomach, but not on the colon (large intestine). Thus, culinary and medicinal consumption of this plant should be monitored.

Acknowledgement

The authors thank every individual who assisted in the successful outcome of this work.

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