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Effect of *Chromlaena odorata* on Histopathological Indices of Intestinal Tissues of albino rats induced with alcohol

Edoga Cyril Onyekachi
Department of Applied Biology and Biotechnology Faculty of Applied Natural Sciences
Enugu State University of Science and Technology

ABSTRACT

Peptic ulcer is an acid-induced lesion of the digestive tract that is usually located in the stomach or proximal duodenum, and is characterized by denuded mucosa with the defect extending into the submucosa or muscularis propria. The aim of this study was to determine the histopathological indices of internal tissues of wistar albino rats induced with alcohol. Twenty five (25) wistar albino rats aged ninety (90) days weighing 150g were used in this study. The rats were randomly selected and grouped into five groups (Group A - E) of five rats per group. Group A, B and C are blank, negative and positive control groups while Group D and E were low and high dose treated groups of the extracts of *Chromolaena odorata respectively*. Twenty-five rats were induced with ulcer using alcohol. After 14 days of treatment, the result showed that the ulcer induced significant elevation of markers of the intestine functions (this can be clearly seen from the decrease in the intestine functioning in the infected and untreated group). The elevation was slightly alternated by treatment in their dose dependent fashion with the most significant effect recorded on group E (high dose).This study also showed that *Chromolaena odorata* has antioxidant agents which helps it to be potent against ulcer, table 1 showed the percentage of ulceration increased significantly (77.674±4.605) when treated with a named ulcer drug, Omeprazole (Group C) while the percentage of ulceration reduced (38.256±6.0847) to a reasonable amount when treated with low dose (Group D) of *Chromolaena odorata.* The treatment with the extracts of *Chromolaena odorata* has an effective, potent antioxidant agent and has the ability to inhibit, reverse and scavenge the reactive oxygen species (ROS) generated by the ulcer infection before reaching the intestine.
Keywords: *Chromlaena odorata,* Histopathological Indices and Intestinal Tissues

INTRODUCTION

The estimated prevalence of peptic ulcer disease in the general population is 5–10% [1,2,3,4,5], but recent epidemiological studies have shown a decrease in the incidence, rates of hospital admissions, and mortality associated with peptic ulcer [6,7,8,9]. This is most likely secondary to the introduction of new therapies and improved hygiene, which resulted in a decline in Helicobacter pylori (*H. pylori*) infections [10,11]. Traditionally, mucosal disruption in patients with the acid peptic disease is considered to be a result of a hypersecretory acidic environment together with dietary factors or stress. Risk factors for developing peptic ulcer include *H. pylori* infection, alcohol and tobacco consumption, non-steroidal anti-inflammatory drugs (NSAIDs) use, and Zollinger–Ellison syndrome [12,13,14]. The main risk factors for both gastric and duodenal ulcers are *H. pylori* infection and NSAID use [15,16]. However, only a small proportion of people affected with *H. pylori* or using NSAIDs develop peptic ulcer disease, meaning that individual susceptibility is important in the beginning of mucosal damage. Almost half of the world's population is colonized by *H. pylori*, which remains one of the most common causes of peptic ulcer disease [17,18,19]. The prevalence of *H. pylori* is higher in developing countries, especially in Africa, Central America, Central Asia, and Eastern Europe [20,21,22]. Peptic ulcer has increased

gastric secretion caused by hypergastrinemia and reduced antral somatostatin content [23,24]. This leads to increased histamine secretion, and subsequently the increased secretion of acid or pepsin from parietal and gastric cells. Additionally, the eradication of *H. pylori* leads to a decrease in gastrin mRNA expression and an increase in somatostatin mRNA expression. In the remaining majority of patients, gastric ulcers are associated with hypochlorhydria and mucosal atrophy [25,26,27,28]. The main mechanism of NSAID-associated damage of the gastroduodenal mucosa is the systemic inhibition of constitutively expressed cyclooxygenase-1 (COX-1), which is responsible for prostaglandin synthesis, and is associated with decreased mucosal blood flow, low mucus and bicarbonate secretion, and the inhibition of cell proliferation [29,30]. The treatment of peptic ulcer begins with the eradication of *H. pylori*. Although successful *H. pylori* eradication alone is paramount for healing associated peptic ulcers and preventing relapses, the growing prevalence of antibiotic resistance made it a global challenge. The first effective therapy was introduced in the 1980s, and consisted of a combination of bismuth, tetracycline, and metronidazole that was given for two weeks [31,32,33]. The standard first-line therapy is a triple therapy consisting of a proton pump inhibitor (PPI) and two antibiotics, such as clarithromycin plus amoxicillin or metronidazole given for seven to 14 days [34,35,36]. However, with an increasing prevalence of antibiotic resistance, especially for clarithromycin, there has been a marked decline in the success of triple therapy over the last 10–15 years. *H. pylori* eradication should be based on antimicrobial susceptibility tests. As susceptibility testing is often not available in clinical practice, the choice of first-line therapies should be based on the local prevalence of antibiotic resistance, and clarithromycin-based regimens should be abandoned in areas where the local clarithromycin resistance rate is more than 15% [37,38]. Levofloxacin triple therapy (PPI, amoxicillin, and levofloxacin) for 14 days seems to be an efficacious therapy, achieving eradication rates between 74–81%. If a patient received first-line treatment with a clarithromycin-based regimen, a preferred treatment option is a bismuth quadruple therapy with eradication rates of 77–93%, or a high-dose dual-therapy regimen with amoxicillin and a PPI, as *H. pylori* rarely develops amoxicillin resistance [39,40]. Despite well-developed recommendations for choosing proper treatment regimens, 5–10% of patients have persistent infection. The most common reasons for the failure of two treatments are suboptimal compliance or the resistance of *H. pylori* to one or more antibiotics, in which case susceptibility testing is strongly recommended. Since up to 13% of patients treated with lansoprazole still experience ulcer recurrence, the search for alternative treatment is ongoing. Vonoprazan is a potassium-competitive acid blocker that inhibits H+, K+-ATPase in gastric parietal cells at the final stage of the acid secretory pathway [17]. The difference in the mechanism of action between vonoprazan and PPIs is that vonoprazan inhibits the enzyme in a K+-competitive and reversible manner, and does not require an acidic environment for activation. Additionally, vonoprazan shows a rapid onset of action and prolonged control of intragastric acidity. Vonoprazan at doses of 10 mg and 20 mg was non-inferior to lansoprazole for the prevention of peptic ulcer recurrence in Japanese patients during NSAID therapy , or those who required aspirin therapy for cardiovascular or cerebrovascular protection [19], with good tolerance, a similar safety profile, and no new safety issues. Also, five weeks of treatment with vonoprazan significantly reduced post-endoscopic submucosal dissection bleeding, compared to eight weeks of treatment with PPIs [22]. Similarly, it was shown to be superior to esomeprazole and rabeprazole for scarring artificial ulcers, which could help make an endoscopic submucosal dissection a safer

treatment. The usage of medicinal plants in healing numerous diseases is as old as human beings, and well-known as phytotherapy. Moreover, in the past few years, there has been a rising interest in alternative therapies and the usage of herbal products, in particular, those produced from medicinal plants [23]. Also, due to appearance of various side effects by usage of conventional drugs for numerous diseases, medicinal plants are considered the major reservoir of potentially new drugs. Plant extracts and their crude are the most significant sources of new drugs, and have been shown to cause promising results in the treatment of gastric ulcer as well [26]. It is known that numerous pharmaceutical agents such as proton pump inhibitors, anticholinergics, antacids, antimicrobial agents, H2-receptor antagonists, sucralfate, and bismuth are not fully effective, and produce numerous adverse effects such as impotence, arrhythmia, hematopoietic alterations, hypersensitivity, and gynecomastia. Due to that, investigations of the new pharmacologically active agents through the screening of different plant extracts led to the discovery of effective and safe drugs with gastroprotective activity. Especially, plants with antioxidant capability such as *Chromolaena odorata* as the main mechanism are used as the herbal reservoir for the treatment of ulcer disease. Medicinal plants have achieved their therapeutic properties from their capability to produce renewable and various secondary metabolites, which are known as phytochemical constituents. Hence, numerous plants have used these phytochemicals as a protection mechanism against pathogens. These plants are also easy to access in endemic areas of ulcer and they are way cheaper than drugs [27].

Justification

Although there has been some breakthrough on the effects of *Chromolaena odorata* on the histopathological indices of intestinal tissues of albino rats but there are dearth of work done on alcohol-induced ulcer. If after this study and *Chromolaena odorata* is proven to be efficient in the treatment of ulcer, people especially the areas it is endemic in will have easy access to it since it is a plant found almost everywhere. And this will reduce cost because it is not bought and it is easily located within geographical settlement thereby providing them with fast access to the treatment of ulcer.

Objective of the Study

The study sought to determine the effects of the oral administration of *Chromolaena odorata* leaves extract on histopathological indices of internal tissues of albino rat induced with alcohol.

MATERIALS AND METHODS

Animal Model and Experimental Procedure

An identical set of 5 groups, consisting of five rats each. Group A was named the blank control group. Here, the rats were neither induced nor treated. Group B was named the negative control. In this group, the rats were induced with 1.0 mg/kg of 97% alcohol to cause ulcer in the rats and they were left untreated. Group C was named the positive control. Here, the rats were induced with 1.0 mg/kg of 97% alcohol and treated with 20 mg/kg of omeprazole. Group D was named low-dose extract. In this group, the rats were induced with 1.0 mg/kg of 97% alcohol and treated with 100 mg/kg of *C. odorata*. Group E was named high-dose. The rats were induced with .0 mg/kg of 97% alcohol and treated with 400 mg/kg of *C. odorata*.

Procurement of Animal

25 male albino rats with an average weight of 150 g were used in this study. Albino rats have been domesticated for purely scientific experiments (Krinke *et al.*, 2000) and differ from wild rats. Albino rats serve as important animal models for researches in both biomedical sciences and psychology (Vandenbergh

2000).The rats were bought from the Animal House, faculty of Basic Medical Sciences, University of Nigeria, Enugu Campus. The animals were housed in wire-gauze cages in a well-lit and adequately ventilated room, under standard environmental conditions (12 hours light and 12 hours dark cycle). They were allowed to acclimatize while being fed with standard laboratory animal chow and water *ad libitum* for 2 weeks.

Sample Collections

At the end of the experiments, the rats were anaesthetized with chloroform inhalation and then killed. Blood samples were collected by heart puncture from each of the rats into well-labeled dry plain tubes for biochemical analysis, while the intestine of the animals were harvested and immediately fixed in 10% neutral buffered formalin for histological studies. After 24 hours of fixation, the intestines were processed immediately via dehydration of tissue in ascending concentrations of alcohol, cleared in xylene, and infiltrated with paraffin wax before embedding. Sections were cut and mounted on slides and stained with hematoxylin and eosin.

Collection of Plant Materials

C. odorata leaves were collected within the locality of in Akpugo in Nkanu Local Government Area of Enugu State, Nigeria and were identified by Prof. C.S. Eze of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Nigeria.

Preparation of Plant Extract

The leaves of *C. odorata* were dried under room temperature with further drying using an oven and ground to fine powder using a manual grinder. Approximately 220 g of the ground sample was weighed using an electronic weighing balance and dissolved in 1000 mL of 75% ethanol. This was properly mixed and allowed to stand for 48 hours, after which it was filtered using Whatman No. 1 filter paper. The filtrate was concentrated by heating in a water bath at 40 °C and the remaining solvent was removed in a rotary evaporator to produce crude ethanolic extract of *C. odorata*.

Statistical Analysis

All statistical analysis was processed using Statistical Program of Social Science (SPSS) software for window. The values of the measured parameters were expressed as mean ± SEM. A one-way Analysis of Variance (ANOVA) was used to determine the effects of alcohol at different doses on wistar albino rats infected with ulcer and the test for significance was recorded as $p< 0.05$.

RESULTS

Percentage Ulceration

The result in Table 1, showed that the percentage ulceration concentration level in the blank control group (0 was significantly decreased ($p<0.05$) when compared with the positive control, low dose and high dose groups (77.674±4.605, 38.256±6.0847, 57.093±2.7952) respectively. The percentage ulcer inhibition in the negative control group was significantly lower ($p<0.05$) when compared with the positive control, low dose and high dose groups. The result showed that the percentage ulcer inhibition of the positive control groups showed the highest significant value. The result also showed that the percentage ulcer inhibition of positive control group increased significantly ($p<0.05$) when compared to the blank group.

Table 1: Protective effect of *Chromolaena odorata* on percentage ulceration of Wistar albino rats induced with alcohol

Groups	Percentage ulceration
A(blank control)	0
B(negative control)	0
C(positive control)	77.674±4.605
D(low dose)	38.256±6.0847
E(high dose)	57.093±2.7952

Along the column, the mean values are also significantly different ($p<0.05$) with a superscript letter.

Table 2: Effects of *Chromolaena odorata* on intestine histology on wistar albino rats induced with alcohol.

Groups	Intestine histology
A(blank control)	0.0525 ± 0.03^{a}
B(negative control)	2.3560 ± 0.40^{d}
C (positive control)	0.1000 ± 0.01^{a}
D (low dose)	1.4420 ± 0.54^{c}
E (mild dose)	0.4200 ± 0.24^{b}

In a column, mean values with the same letter as superscript are not significantly different ($p>0.05$).

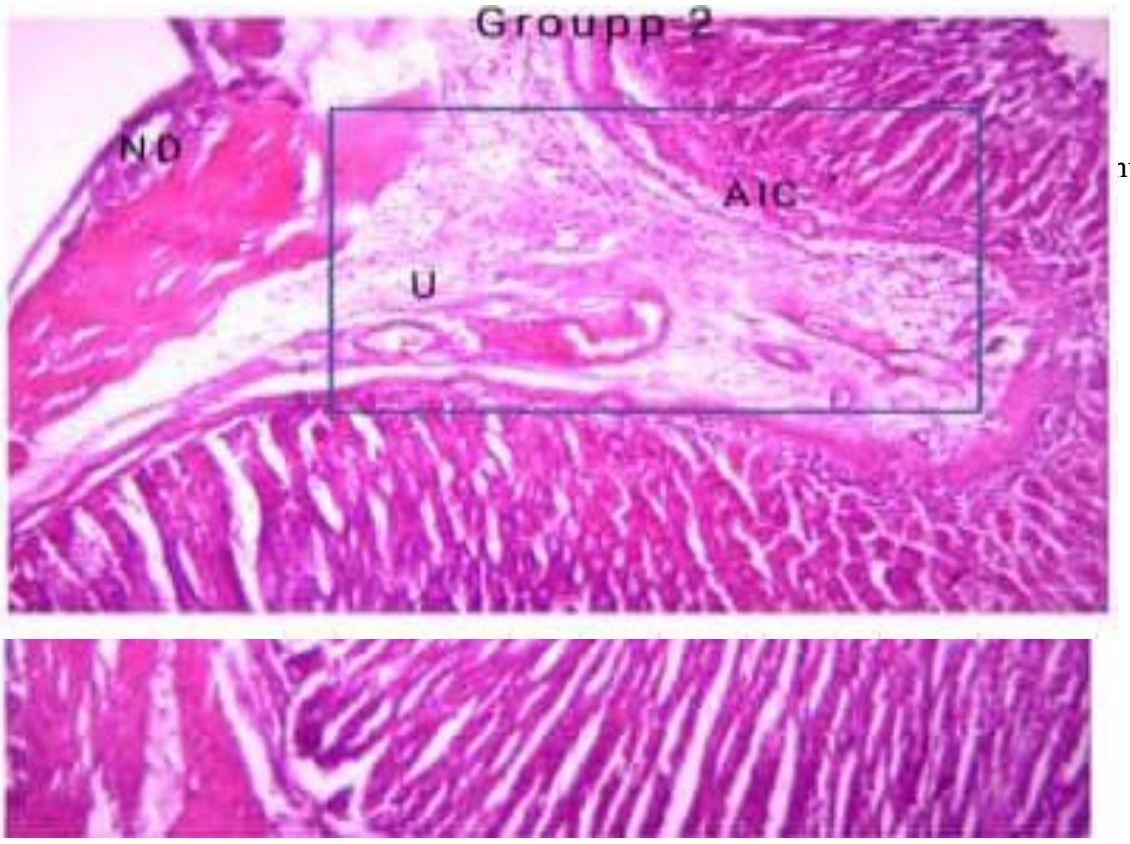

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Plate 1: Photomicrograph of Group A (×150) (H/E)
Negative control (Group B)
Sections of intestine collected from the animal in group (negative control) induced with ulcer and left untreated showed moderate to severe effects on the lining of the intestine with severe ulceration (U) and moderate aggregate of inflammatory cell (AIC) within the ulcerated area at sub mucosa layer and mild necrotic debris (ND) within the muscularis externa.

Plate 2: Photomicrograph of Group B (×150)(H/E).

Positive control (Group C)

Section of the intestine collected from the animals in this group (positive control) infected with ulcer and treated with omeprazole showed moderate healing with mild ulceration (U) within the sub mucosa.

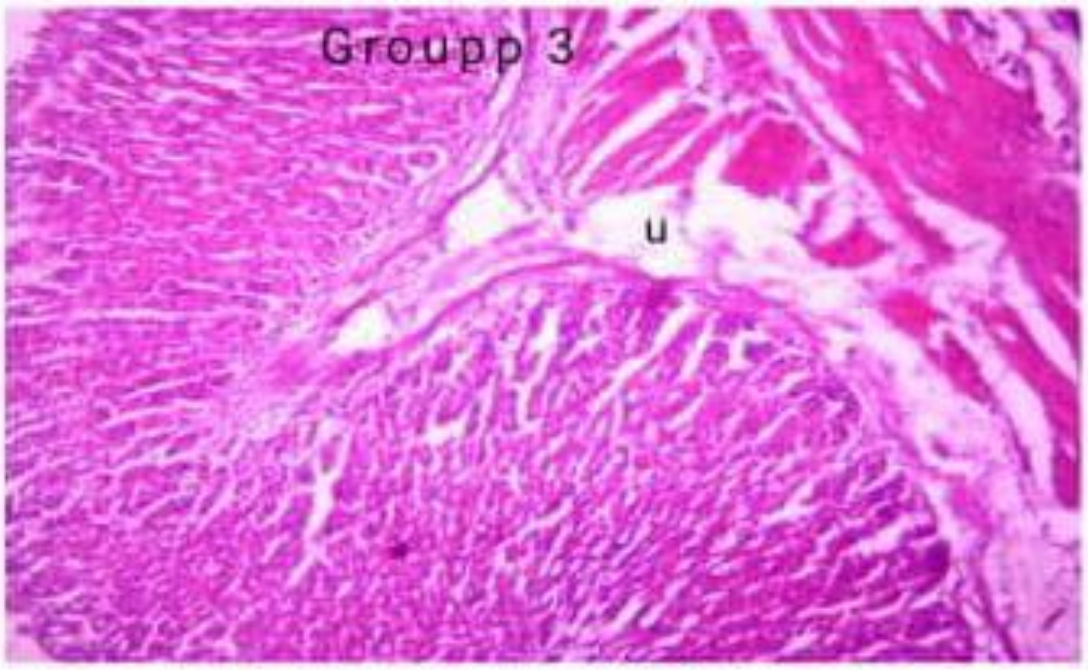

Plate 3: Photomicrograph of Group C (×150)(H/E).

Low-dose Omeprazole (Group D)

Sections of the intestine collected from the animals in this group (low dose of extract) induced with 1.0 ml/kg of alcohol and treated with 100 mg/kg of *Chromolaena odorata* showed that mild to moderate healing with mild ulceration (U) and moderate aggregate of inflammatory cell (AIC) within the sub mucosa.

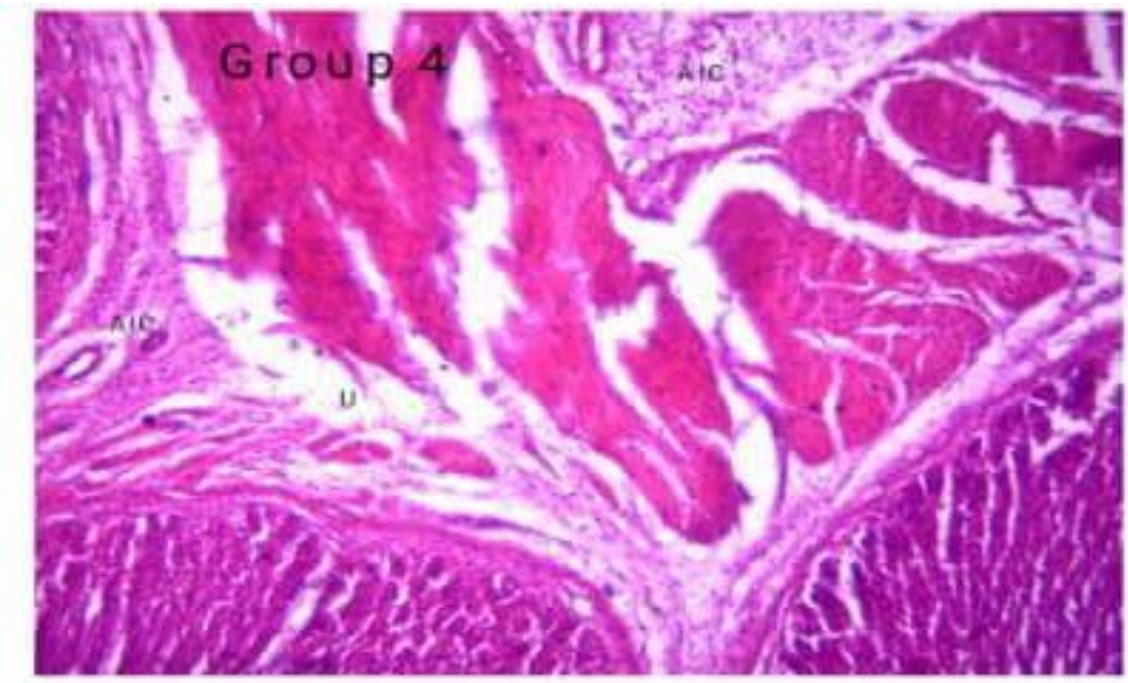

Plate 4: Photomicrograph of Group D (×150) (H/E).

High-dose of Omeprazole (Group E)

Sections of the intestine collected from the animals in this group (high dose of extract) induced with 1.0 ml/kg of alcohol and treated with 400 mg/kg of *Chromolaena odorata* extract showed moderate healing with mild ulceration

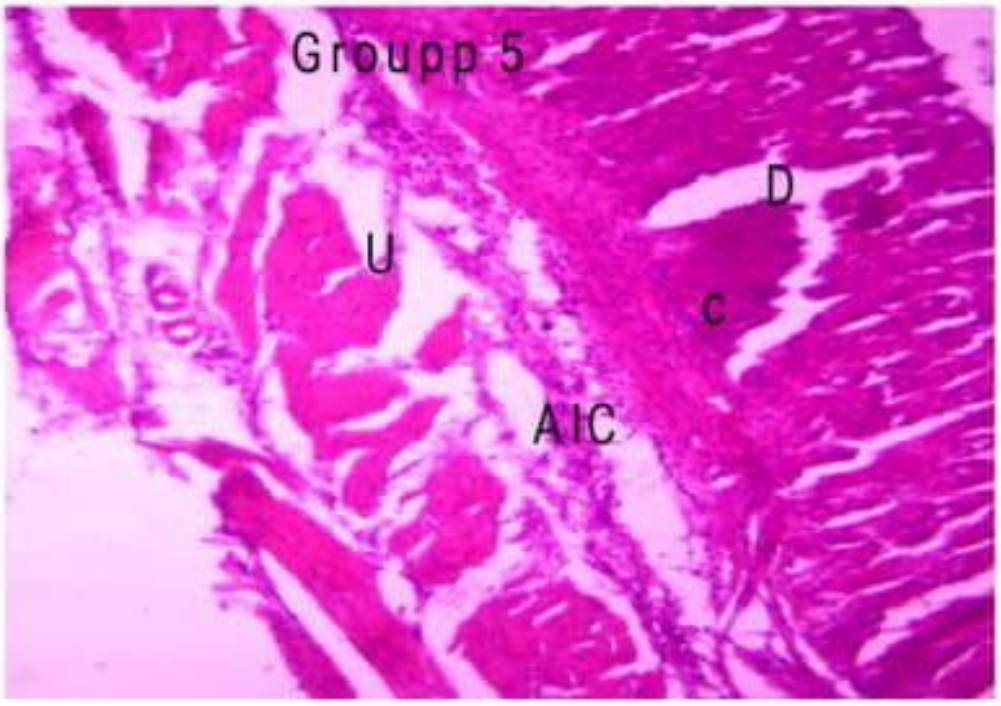

Plate 5: Photomicrograph of Group D (×150) (H/E).

DISCUSSION

Quantitative analysis of *Chromolaena odorata* leaves extract on histopathological indices of internal tissues of albino rat induced with alcohol revealed the presence of phytochemicals as a protection mechanism for ulcer treatment. The values of the plant extract are expressed per g.The effects of the leave extract of sub mucosa (SM) and mucosaris externa (ME) on the ulcer index and % inhibition against ulcer in the experimental animals are shown above in plates 1 and 2 respectively. Oral administration of 1ml/kg b.w. of the alcohol extract from the *chromolaena odorata* leaf caused a significant ($p < 0.05$) increase in the degree of the ulceration (ulcer index) in the rats. A significant improvement in the level of inhibition against ulceratiom was however observed in the extracts treated animals. The extract at 200 mg/kg b.w. offered better protection against ulceration than the 100 mg/kg b.w. regimens and compared well with the standard drug (Omeprazole) used. Plate 3 shows the effect of *Chromolaena odorata* leaf extracts of SM and ME on gastric secretions of Indomethacin ulcerated rats. From the plate, the administration of Indomethacin caused significant ($p < 0.05$) decrease in PH value scale with a corresponding significant ($p < 0.05$) increase in gastric volume of gastric content. The pre-treatment also resulted to a significant increase in pH value coupled with significant decrease in gastric volume when compared with ulcerated control rats. Plate 4 shows the *Chromolaena odorata* administration also brought about a significant ($p < 0.05$) increase in specific pepsin activity as well as significant reduction ($p < 0.05$) in mucin content of gastric juice of ulcerated rats when compared with the normal control. The observed changes in these parameters were significantly attenuated ($p < 0.05$) in the SM and ME extract treated rats. Plate 5 revealed the effects of the *Chromolaena odorata* leaf extracts of SM and ME on the phytochemicals administered on the albino rats. The phytochemical level was significantly increased ($p < 0.05$) in the ulcerated animals (albino rats). It can be observed that both extracts particularly at 200 mg/kg b.w. resulted in significant improvement ($p < 0.05$) in these parameters and the observable effects

compared favorably well with both normal control and standard drug.

CONCLUSION

The result of the present study showed that the ulcer induced significant elevation of markers of the intestine functions (this can be clearly seen from the decrease in the intestine functioning in the infected and untreated group). The elevation was slightly alternated by treatment in their dose dependent fashion with the most significant effect recorded on group E (high dose). The result also shows that the extracts of *Chromolaena odorata* has an effective, potent antioxidant agent and has the ability to inhibit, reverse and scavenge the reactive oxygen species (ROS) generated by the ulcer infection before reaching the intestine.

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