International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 4, Issue 2, pp: (1141-1149), Month: October 2016 - March 2017, Available at: www.researchpublish.com

Therapeutical Effect of Crude Extract of *Garcinia Kola* on Acetic Acid-Induced Ulcerative Colitis in Male Rats

M.J. Adeniyi[1], S.F. Ige[2]

[1]Department of Physiology, University of Benin, Benin-city
[2] Department of Physiology, Ladoke Akintola University of Technology Ogbomoso

Abstract: **We previously reported that pretreatment with *Garcinia kola* (GK) crude extract alleviated hyperthermia in colitis and improved intestinal antioxidant enzymes in both healthy and colitis male rats. The present study investigated the therapeutical effect of the extract on acute acetic acid induced ulcerative colitis in male wistar rats. 20 male wistar rats weighing 120g-150g were randomly divided into vehicle- treated, *Garcinia kola*, colitis and colitis treated groups. Acute colitis was induced with 1ml/200g of acetic acid and 150mg/kg (P.O) was administered once per day as previously reported. The result showed that treatment with GK did not prevent weight loss 5 days posttreatment. Significant increase (P<0.05) was noticed 14 days and 21 days posttreatment.When compared with colitis group, colitis treated group showed a significantly low (P<0.05) diarrhea and ulcer scores. Also, tissue thickness of colitis treated group was significantly reduced (P<0.05). Histological studies of the colon tissues of colitis treated rats revealed regenerating mucosal layer with numerous regenerating colonic crypts and a distinguishable colonic layers.The findings of this study suggest that GK exerted a therapeutical effect on acute acetic acid induced colitis in male wistar rats.**

Keywords: **Garcinia kola, colitis, acetic acid, colon, therapeutical, colon.**

1. INTRODUCTION

Ulcerative colitis is one of the diseases of the large intestine (Ford *et al.*,2013). It is commoner in North America and Europe affecting 5 to 500 per 100,000 individuals (Danese and Fiocchi 2011). In Africa, the first case of ulcerative colitis was reported in an eight year old Nigerian girl in 2012 presenting with blood stained stool, abdominal pain and significant weight loss (Senbanjo *et al.*,2012).

Ulcerative colitis usually affects the lining of the large intestine and its cause remains unknown. Interactions among genetic, immune and environmental factors have been suggested (Roendinger ,1980; Rachimetz, *et al*.,1997; Cummings ,*et al*. ,2003) . In animal model and experimental studies using rats, acetic acid (Adeniyi *et al.*,2012), ethanol, indomethacin and dextran sulphate sodium (Farombi *et al*.,2013) have used to induce ulcerative colitis

In animal model of inflammatory bowel disease, acetic acid - induced mucosa damage caused pathological changes such as mucosal ulceration, weight loss, bloody stool (Ohkusa , *et al*.,2003) , inflammatory responses such as neutrophil infiltration, increased mucosal production of myeloperoxidase , leukotriene (Rachimilewitz ,*et al.*,1989) upregulation of cyclooxygenase and nitric oxide synthetase activity (Rachimilewitz ,*et al.* ,1995).

As far as dietary management of the ulcerative colitis is concerned, reduction in alcohol and meat intake (Jowett *et al.*,2004), increase intake of antioxidants such as vitamins A, C and E, manganese and selenium and increase in consumption of plant fiber (Wright and Truelove 1965) have been devised. Studies have shown that extracts from plants demonstrates potentials of alleviating symptoms of ulcerative colitis (Olaleye and Farombi 2006; Faromb *et al*.,2013; Adeniyi *et al.*,2016). For instance, Ige *et al.*,(2012) reported that methanolic extract of *Garcina kola* improved gastric healing and reduced gastric damage induced by acute ethanol instillation in male rats. We also reported in our previous

study that pretreatment of colitis rats with *Garcinia kola* crude extract alleviated hyperthermia and improved intestinal antioxidant enzymes (Adeniyi *et al.*,2016). The present study was designed to determine the therapeutical effect of the extract on acetic acid induced colitis in male rats.

2. MATERIALS AND METHODS

SITE OF THE STUDY:

The experiment was carried out at the animal house, Physiology Department in Ladoke Akintola University Ogbomosho Oyo State. The histological analysis was done in Pathology department of University of Ilorin , Kwara state.

ANIMAL CARE AND MANAGEMENT:

Twenty adult male wistar rats weighing 120-150g were used for the research work. They were divided into four groups consisting of five animals each. These rats were kept in five different cages with a wire mesh covering .They were fed pelletized grower's mash adlibitum, provided water through drinking trough and kept under 12 hour light and 12 hour darkness at room temperature.

ETHICAL CERTIFICATION:

The study was conducted in line with the guidelines of National Institute of Health (NIH) for the use of laboratory rats.

EXPERIMENTAL PROCEDURE:

The rats were weighed and randomly grouped into;

Group A: received distilled water for three weeks and was designated as Vehicle-treated group.

Group B: received *Garcinia kola* extract for three weeks and was designated as *Garcinia kola* group.

Group C: received distilled water for three weeks then acetic acid was infused and was designated as colitis group

Group D: received a single intracolic acetic acid instillation and were treated with *Garcinia* kola for three weeks and was designated as colitis treated group.

METHODS:

EXTRACTION OF GARCINIA KOLA:

The *Garcinia kola* seeds were obtained commercially from the Oja Igbo in Ogbomosho. The outer coats were removed and the seed cut into pieces and air died. The dried seeds were ground to fine powder and methanolic extraction was done by a soxhlet extraction. The yield was concentrated to a semi-solid form. 3.0g of the extract was measured and dissolved in 3% ethanol to give 30mg/ml.

ADMINISTRATIONS:

Distilled water and 150mg/kg of *Garcinia kola* extract (P.O) was administered for three weeks once per day.

INDUCTION OF COLITIS: After fasting the animals for 24 hours , the animals were anasthesized with a light ether anesthesia, a flexible plastic catheter (outer diameter = 2mm) was inserted rectally into the colon (catheter was placed about 8cm proximal to the anus). Colitis was then induced by administering 1ml/200g of 7% acetic acid.

DIARRHEA SCORING:

The scoring of the diarrhea was done according to the method of Masonobu *et al.*, (2002), with little modification. Scoring of diarrhea was done 24 hours after the induction of colitis.

Table 1: Diarrhea scoring method (Masonu *et al.*,2002)

SCORE	OBSERVATION
0	Normal stool
1	Loose stool without visible blood
2	Visible blood
3	Loose stool with visible blood
4	Bloody diarrhea

SACRIFICE AND ASSESSMENT OF COLON:

All animals were sacrificed at the end of the experiment. After sacrificing the animals through cervical dislocation, the distal colon, (8cm) was removed and opened longitudinally, washed to remove luminal contents with normal saline. The thickness of the colon (in mm) was measured with meter ruler.

ULCER SCORE METHOD:

Table 2: Ulcer scoring method (Zheng and Wang 2000)

SCORE	OBSERVATION
0	Normal damage
1	Localized hyperemia with no ulcer
2	Linear ulcers with no significant inflammation
3	Liner ulcer with inflammation at one site
4	More site of ulcers and inflammation, the size of ulcer < 1cm
5	Multiple inflammations and ulcers, the size of ulcer >1cm.

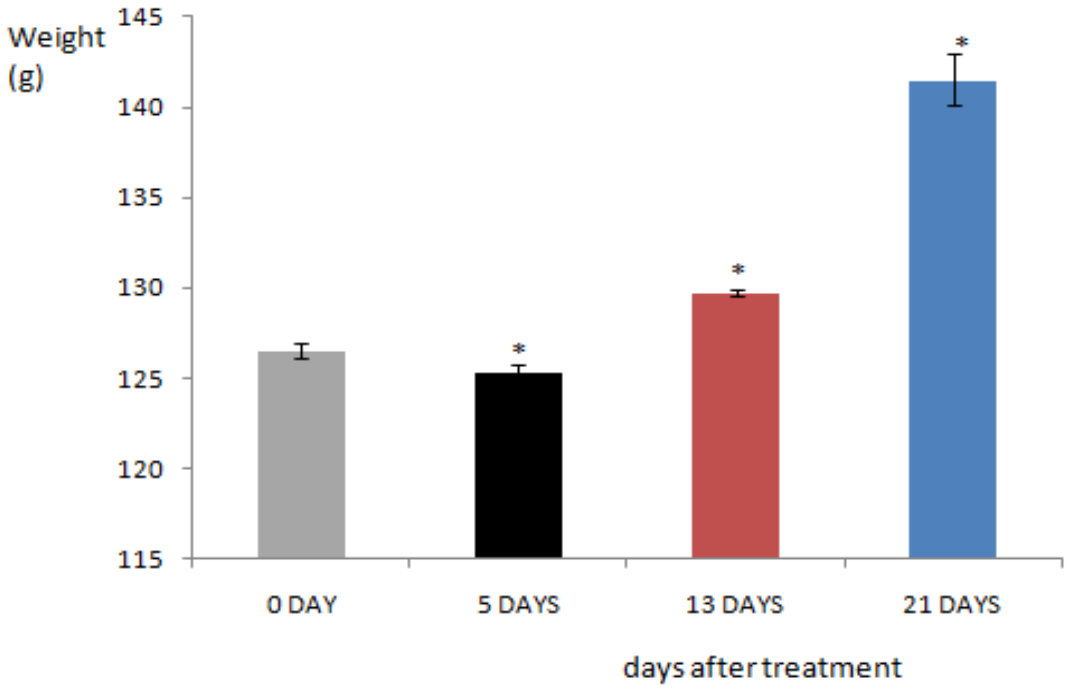
After the colon was removed and opened longitudinally, the excised tissue was pinned out and washed with 0.9 % normal saline .With the mucosa side up on a wax platform , visible damage was examined with the aid of magnifying lens and scored on a 0-5 scale (Zheng and Wang 2000)

PREPARATION FOR THE EXPERIMENT:

After macroscopic study, the colon was immersed in formalin to be preserved for histological. **HISTOLOGICAL ANALYSIS:**

After fixing these colons with 10 % formalin, they were transferred to 70 % alcohol for dehydration .After 6 hours they were transferred to 90 % alcohol and left overnight .They were then transferred to xylene for about 10 hours and later transferred to a fresh xylene for 30 minutes each in an oven at 570c. Apoptotic cells were identified by the H & E staining.

3. RESULT



** significant difference (P<0.05) from 0 day*

Figure 1: Weight response of colitis rats to GK

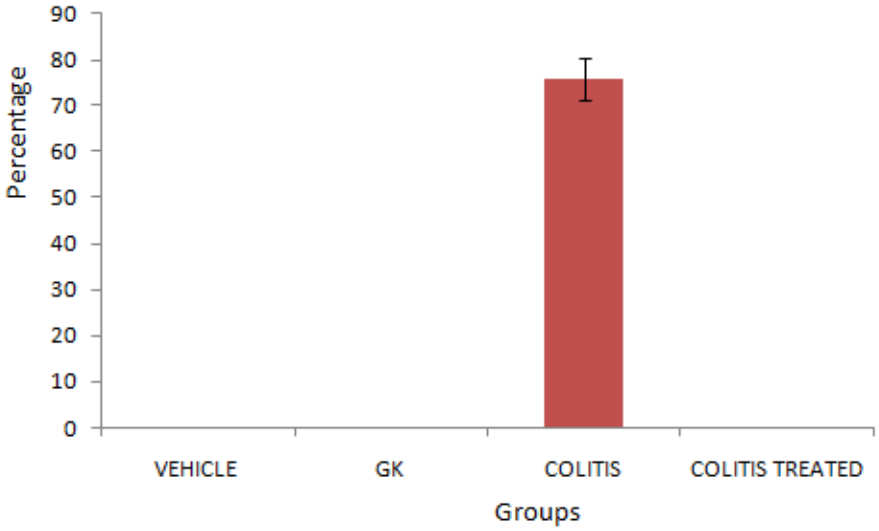
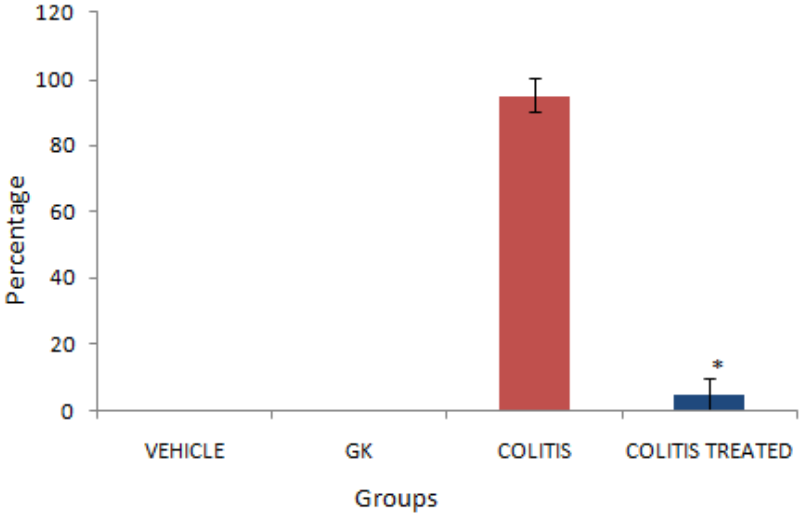
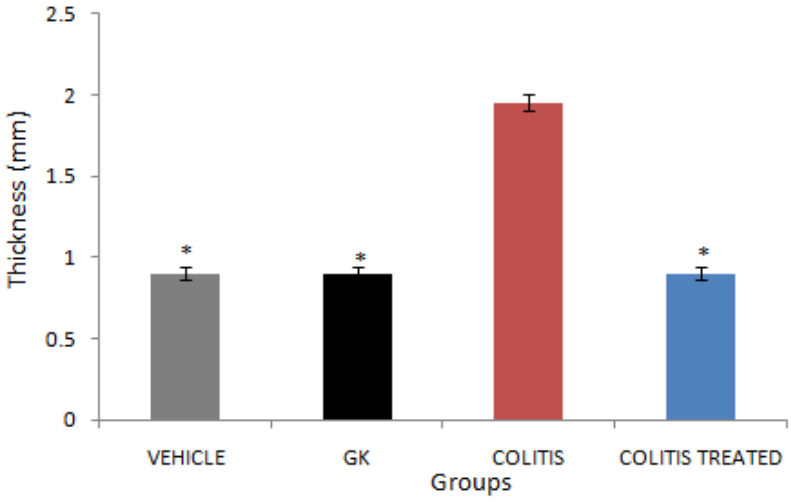


Figure 2: Diarrhoea score



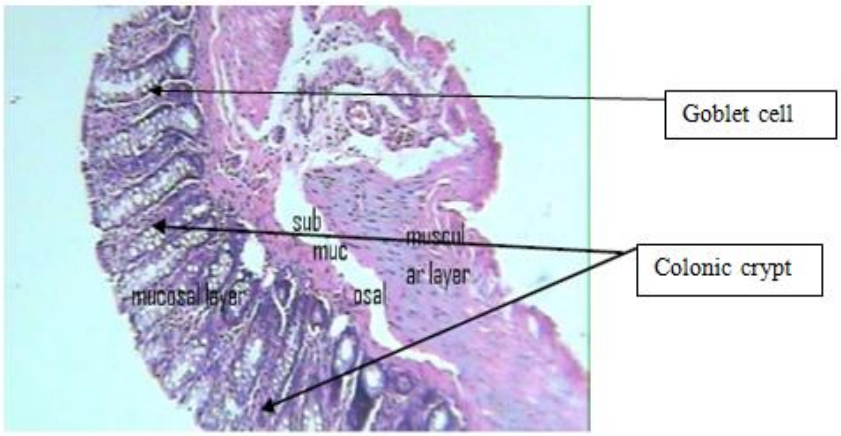
** significant difference (P<0.05)from colitis group*

Figure 3: Ulcer score



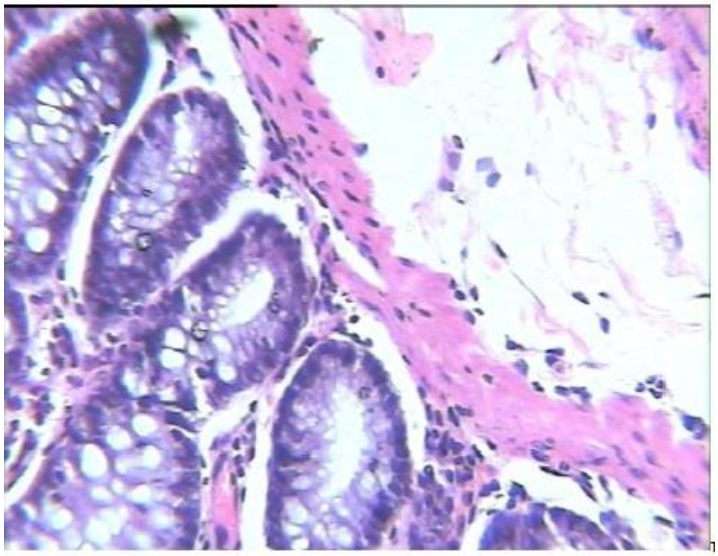
** significant difference (P<0.05) from colitis group*

Figure 4: Tissue thickness



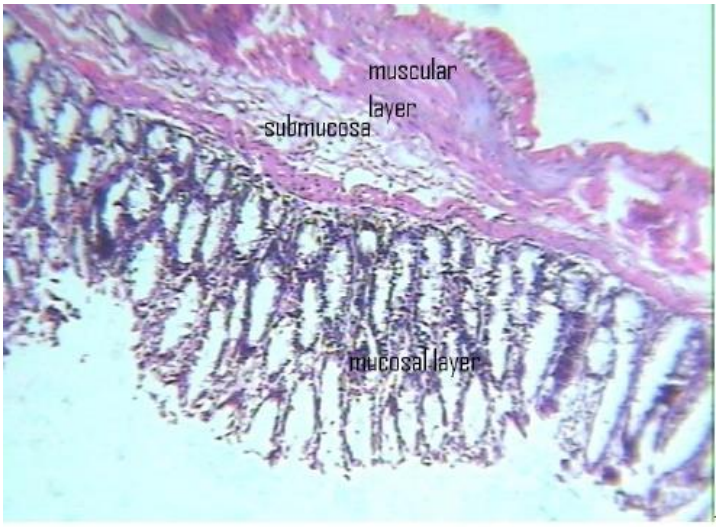
(H&E STAIN)

Figure 5A: colonic histology of vehicle treated rats under X100 showing colonic crypts, goblet cells and distinguishable layers.



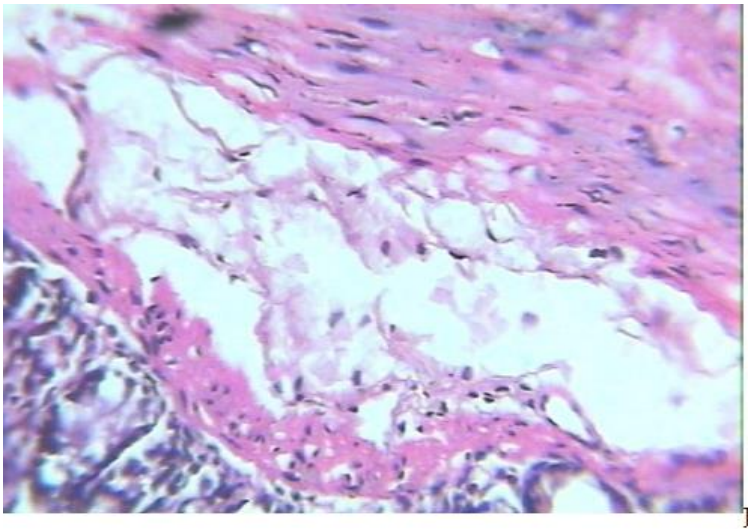
(H&E STAIN)

Figure 5B: colonic histology of vehicle treated rats under X400.



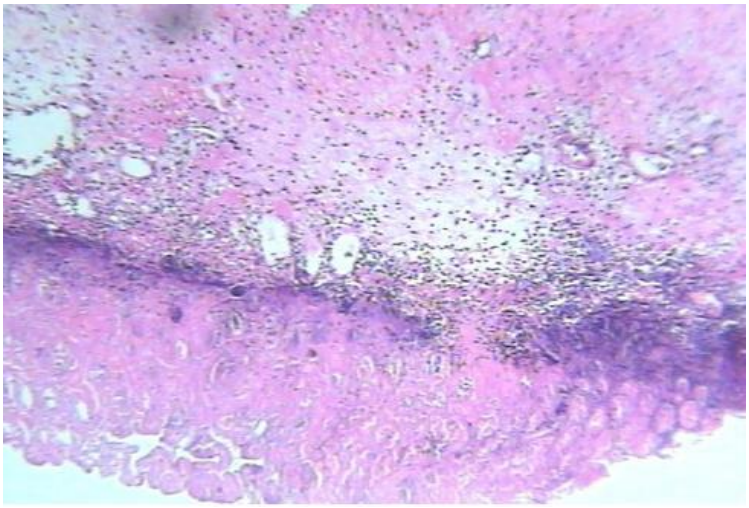
(H&E STAIN)

Figure 6A: Colonic histology of Garcinia Kola treated rat under X100



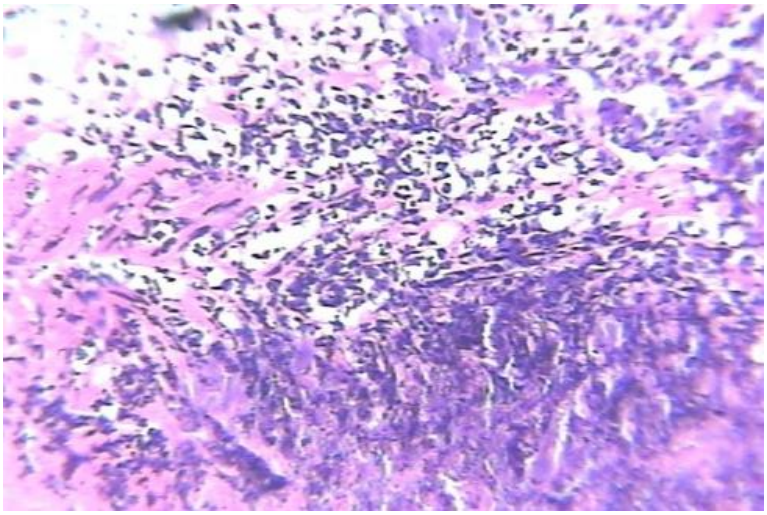
(H&E STAIN)

Figure 6B: Colonic histology of Garcinia Kola treated rat under X400



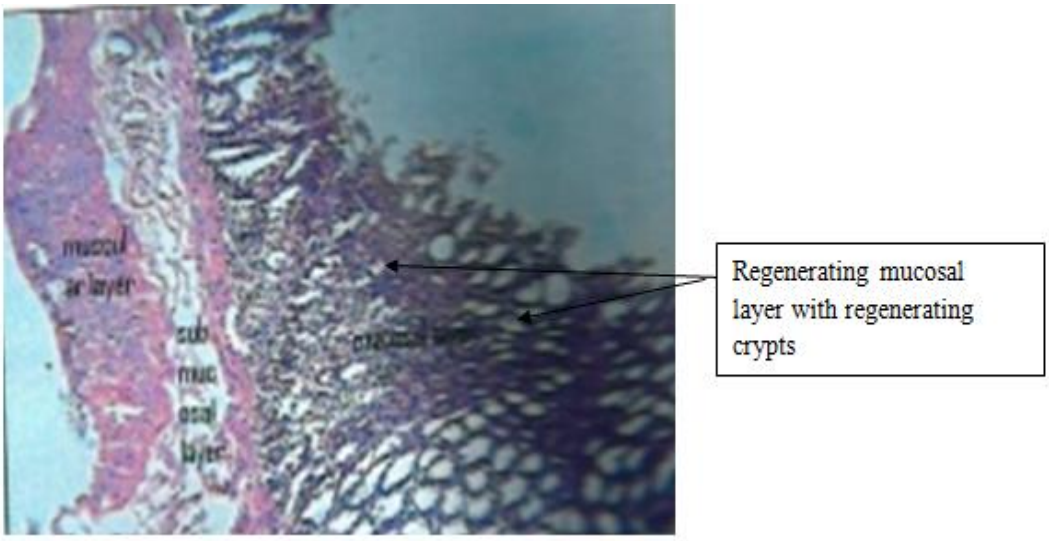
(H&E STAIN)

Figure 7A: Colonic histology of colitis rats under X100. No distinguishable colonic layers



(H&E STAIN)

Figure 7B: Colonic histology of colitis rats under X400 showing extensive leucocyte infiltration



(H&E STAIN)

Figure 8A: Colonic histology of colitis treated rats under X100 showing regenerating mucosal layer with regenerating crypt and distinguishable colonic layers

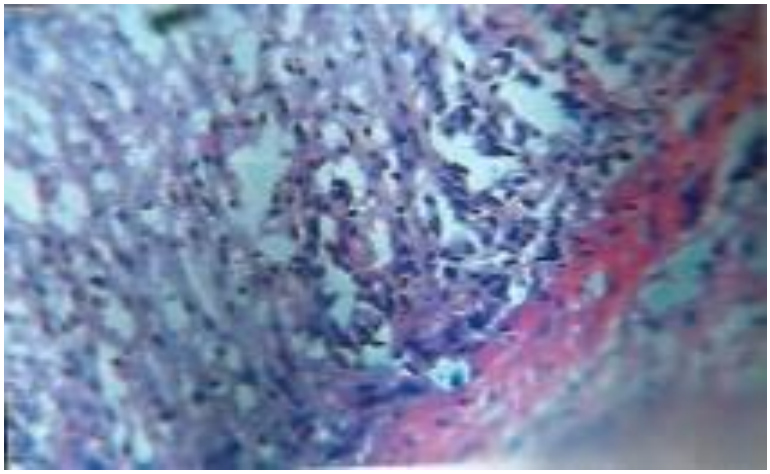


Figure 8B: Colonic histology of colitis treated rats under X400

4. DISCUSSION AND CONCLUSION

Ulcerative colitis is a disease in which the colon loses its structural integrity and the ability to absorb water and form faeces (Rubin *et al.*,2005). The findings of this study corroborated the fact that ulceration, loss of structural components of colonic tissue and diarrhea are characteristic features of human and experimental model of ulcerative colitis (Ohkusa *et al.*,2003; Ford *et al.*,2013).

Treatment of colitis rats with the methanolic extract of *Garcinia kola* in this study reduced ulcer score. *Garcinia kola* contains biflavonoids (Iwu and Igboko 1982) and biflavonoids have been shown to stimulate angiogenesis, an important facet of tissue healing (Kilicoglu *et al.,* 2008). The histological studies of colitis treated rats reveal regenerating mucosal layer with regenerating colonic crypts and goblet cells (Figure 8A) in contrast to the histology of colitis rats which are characterized by indistinguishable colonic layers (Figure 7A).

We also observed that *Garcinia kola* extract ameliorated diarrhea score. Compounds containing biflavonoids could inhibit development of fluid that results in diarrhea by targeting cystic fibrosis transmembrane conductance regulator (CFTR) of chloride transport inhibiting cAMP-stimulated chloride ion secretion in the intestine (Thiagarajah and Verkman 2012).

Swelling is one of the symptoms of inflammation. The result of the study indicated that treatment of colitis rats with methanolic extract of *Garcinia kola* decreased tissue thickness. A studies by Olaleye *et al.*,(2000) and Olaleye and

Farombi (2006) revealed the anti-inflammatory potency of flavonoid -rich kolaviron. Flavonoid rich compounds have been reported to reduce the release of histamine, bradykinin and leukotriene which could cause an increase in vascular permeability and escape of macromolecules from microcirculation (Sy *et al.*,2006; Epub 2006). This reductive effect results into reduction in size of edema, leucocyte infiltration, and DNA damage in isolated colonocyte (Tan-No *et al.*,2006).

The cumulative result of antiulcer, antiinflammatory and antidiarrhea effects of the extract of *Garcinia kola* was the decrease in weight loss 13days and 21 days after treatment of colitis rats. Since colon is heavily colonized with microorganism, regulation of microbial activities by flavonoid containing compounds including *Garcinia kola* might have prevented the from worsening. We also noticed that *Garcinia kola* extract did not prevent weight loss in the first week of treatment. In a study by Natusforsch (1996), seven days of treatment after experimental gastritis with flavonoid containing compound did not assuage symptoms of disease.

The present study showed that treatment of colitis rats with *Garcinia kola* extract decreased ulcer score by stimulating colonic healing and reduced diarrhea score and tissue thickness. The extract also improved weight of colitis rats thirteen and twenty one days after treatment. Therefore, methanolic extract of *Garcinia kola* exhibited therapeutical effect.

ACKNOWLEDGEMENT

We are grateful to the laboratory staffs of Department of Physiology for their support and cooperation during the course of this research.

Conflict Of Interest: Nil

REFERENCES

[1] Adeniyi M.J., S.F. Ige, W.J. Adeyemi, T.J. Oni, P.O. Ajayi, S.O. Odelola, and V.T. Adegbola. (2016). “Changes In Body Temperature and Intestinal Antioxidant enzymes In Healthy And Colitis Male Rats: Roles Of *Garcinia kola*”. *International Journal of Physiology*; 4(2): 36-41.

[2] Cummings JH, Banwell JG, Segal L Coleman, Englyst HN and Macfarlane GT (1990). ‘The amount and composition of large bowel content in man’. *Gastroenterology*; 98: 408.

[3] Cummings JH, Macfarlane GT and Macfarlane S (2003). ‘Intestinal Microbiology’. *Curr Issues intest Microb*; 4: 9-20.

[4] Danese, S; Fiocchi, C (3 November 2011). ‘Ulcerative colitis.’ *The New England journal of medicine*; 365 (18): 1713–25.

[5] Epub Camacho Barquero(2006). ‘Effects of cucumin on chronic colitis’. *Int. J Immunopharmacol*; 7(3):333-42

[6] Farombi Olatunde, Ajayi Oluwaseun Babajide, Isaac A. Adedara and Omolola R. Ayepoola (2013). ‘Kolaviron, A Natural Antioxidant and Antiinflammatory Phytochemical Prevents Dextran Sulphate Sodium- Induced Colitis in Rats’. *Basic and Clinical Pharmacology and Toxicology*; 113 (1).

[7] Ford, AC; Moayyedi, P and Hanauer, SB (5 February 2013). ‘Ulcerative colitis.’ *BMJ*; 346: 432.

[8] Ige S.F, Akhigbe R.E, Olaleye S.B and Adeyemi J.W (2012). ‘Gastroprotective potentials of the methanolic extract of *Garcinia kola* in rats’. *Int J Med Biomed Res* ;1(3):172-178 172

[9] Iwu MM and Igboko O (1882). ‘Flavonoids of *Garcinia kola* seeds.’ *Nat. Prod*; 45: 650-651. Jowett, S L; Seal, C J; Pearce, M S; Phillips, E; Gregory, W; Barton, J R and Welfare, M R (2004). "Influence of dietary factors on the clinical course of ulcerative colitis: a prospective cohort study". *Gut.* 53 (10): 1479–1484.

[10] Kilicoglu Sibel Serin, Bulent Kilicoglu and Ezra Erdemli (2008). ‘Ultrastructural view of colon anastomosis under Propolis effect by transmission electron microscopy’. World J Gastroenterology; 14(30): 4763-4770.

[11] Masonobi F, Osamu K, Yoshio A, Akira A, Kehchi M, and Kohsuke T, *et al.*,(2002). ‘Prebiotic treatment of experimental colitis with germinated barley food stuff: A comparison with probiotic or antibiotic treatment’. Int J Molmed; 9: 65-70.

[12] Natusforschz (1996). ‘Healing process induced by a flavonoic fraction of Biden aurea.’ 51 (7): 570-7.

[13] Ohkusa T, OkayasuI, Ogihara T and Morita K (2003). 'Induction of experimental ulcerative colitis by Fusobacterium Varium'. 52 (1): 79-83.

[14] Olaleye SB and Farombi EO (2006). 'Attenuation of indomethacin-and HCl/ethanol-induced oxidative gastric mucosal damage in rats by Kolaviron. *Phytother. Res*; 20: 14-20.

[15] Olaleye SB, Farombi E, Adewoye EA, Owoyele EA, Onasanwo SA and Elegbede RA (2000). 'Analgesic and anti-inflammatory effect of kolaviron; Africal Journal of Biomedical Research; 3 (3): 171-174.

[16] Rowe, A. H and Rowe, A. (1960-07-01). 'Chronic ulcerative colitis--atopic allergy in its etiology'. *The American Journal of Gastroenterology. 34: 49–66.*

[17] Rachimilewitz D, Simon PL, Schwartz LW, Griswold DE, Fondacaro JD *et al.*,(1989). 'Inflammatory mediators of experimental colitis in rats' *Gastroenterology*; 97: 326-327.

[18] Rachimilewitz D, Stamier JS, Karmeli F, Mullin ME, Singel DR *et al.*,(1995). 'Enhanced colonic nitric oxide synthetase activity in ulcerative colitis and chrohn's disease'. *Gut*; 36: 718-723.

[19] Roendinger WEW (1980). 'The colonic epithelium in ulcerative colitis: an energy-deficiency disease', *Lancet*; 11:712-15.

[20] Rubin Emmanuel, Fred Gorsten and Roland Schwarting David (2005). 'Rubin Pathology'. 4th ed. Philadelphia: 355-366.

[21] Thiagarajah JR and Verkman AS (2012). 'CFTR inhibitors for treating diarrheal disease'. *Clinical Pharmacology and Therapeutics*; 92 (3): 287-90.

[22] Senbanjo Idowu O, Kazeem A Oshikoya, Charles A Onyekwere, Fatimah B Abdulkareem and Olisamedua F Njokanma (2012). 'Ulcerative colitis in a Nigerian girl: A case report'. *BMC;* 5:564.

[23] Sy LB, Wu YL, Chiang BL, Wang YH, Wu WM (2006). 'Propolis extract exhibits an immunoregulatory activity in an OVA-sensitized airway inflammatory animal model'. *Int Immunopharmacol*;6: 1053-1060.

[24] Tan-No K, Nakajima T, Shoji T, Nakagawasai O, Niijima F, Ishikawa M, Endo Y, Sato T, Stoh S, and Tadano T, (2006). 'Anti-inflammatory effect of Propolis through inhibition of nitric oxide production on carrageenin-induced mouse paw edema'. *Biol. Pharm. Bull*; 29: 96-99.

[25] Zheng L, Gao ZO and Wang SX (2000). ' A chronic ulcerative colitis model in rat'. *World Journal of Gastroenterology*; 6(1): 150-152.

[26] Wright, Ralph; Truelove, S. C. (1965-07-17). 'A Controlled Therapeutic Trial of Various Diets in Ulcerative Colitis'. *British Medical Journal.* 2 (5454): 138–141.