Therapeutic Potential of Aqueous Seed Extract of *Garcinia Kola* on Experimental Colitis Induced by Acetic Acid Colitis in Wistar Rats

Abstract

Introduction: Erosive colitis is a common disorder of the gastrointestinal tract with unknown aetiology characterized by recurrent erosion of the colonic mucosal lining. The pathogenesis likely involves genetic, environmental, and immunologic factors.
Aim: This study investigated the therapeutic effects of *Garcinia kola (GK)* on acetic acid-induced erosive colitis in adult male Wistar rats. Erosive colitis (EC) was induced in male Wistar rats by intra-rectal administration of 2 ml of 4 % acetic acid at 8 cm proximal to the anus for 30 seconds.
Methodology: Thirty-six animals were divided into six groups (n=6), negative control (no erosion, no GK); positive control (erosive colitis, no GK); treated with 100, 200, 400mg/kg GK, standard treatment group (Prednisolone) for a period of 14 days.
Results:GK significantly improved EC-induced reduction in mean stool consistency and mean macroscopic erosion score ($P < 0.05$). Injection of GK also significantly reduced the mean microscopic erosion score when compared to untreated EC control ($P < 0.01$). GK reduced the colonic mucosa injury of the wistar rat.
Conclusion: Results of this study provide scientific evidence which lend credence to the use of aqueous seed extract of *Garcinia kola* in the folklore medicine in the management of colitis erosion.

Keywords: Erosive colitis, *Garcinia Kola* and Wistar Rats

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1.0INTRODUCTION

Erosive colitis is a disease condition in which the colon loses its structural integrity and the ability to absorb water and form faeces[1].Patients with erosive colitis may present with symptoms such as watery diarrhoea, abdominal pain, tenesmus, urgency, fever, subjective fatigue, or blood in the stool [2].There are several different causes of erosive colitis, including infection, autoimmunity, ischemia, toxin exposure, immunodeficiency, and radiation exposure [3]. Erosive colitis is a common and increasing disease incidence worldwide. Nearly one million individuals each in the United States and Europe are affected by this condition and many more globally. Over the past decade, since the publication of the last guideline from the American College of Gastroenterology (ACG) on this topic, it occurs throughout the world but is more common in urban areas and present in teens at early 20s [4]. Despite the fact that aetiology of erosive colitis still remains poorly understood, complex interactions among genetic, environmental, immunological and reactive oxygen species (ROS) have been implicated in the pathogenesis of erosive colitis [5];[6].

Comment [DP5]: This term needs to be clearly defined. Is it similar to ulcerative colitis or inflammatory bowel disease or a new entity?

Garcinia kola is a species of flowering Plant which belongs to a family of tropical plants known as Clusiaceae or Guttiferae also known as African wonder nut. In Nigerian languages, it is commonly called *Namijingoro* in Hausa, *Agbilu* in igbo, and *Orogbo* in Yoruba. Garcinia kola has economic and cultural values across West and Central African countries where the nuts are commonly chewed and used for traditional ceremonies [7].The seeds are also used in folk medicine in many herbal formulations and have potential therapeutic benefits due largely to the activity of their flavonoids and other bioactive compounds [8]. This plant has been referred to as a "wonder plant" because every part of it has been found to be of medicinal importance The seeds are chewed as an aphrodisiac or used to cure cough, dysentery, chest colds, liver disorders, diarrhoea, laryngitis, bronchitis, and gonorrhea [9]. The seed is used to prevent and relieve colic; it can also be used to treat headache, stomach ache and gastritis [10]

1.1 STATEMENT OF THE PROBLEM

Erosive colitis is a common gastro intestinal disorder. Although there are few epidemiologic data conducted in developing countries like Nigeria but it has an increasing incidence worldwide. Nearly one million individuals each in the United States and Europe are affected by this condition and many more globally. Over the past decade, since the publication of the last guideline from the American College of Gastroenterology (ACG) on this topic, epidemiological studies from all over the world have stated that the incidence and prevalence of erosive colitis are increasing with time and in different regions around the world indicating its emergence as a global disease [11].

1.4 JUSTIFICATION OF THE STUDY

Acetic acid induced erosive colitis is a commonly employed and easily inducible model of erosion. Intra rectal administration of dilute solution of acetic acid causes non-transmural erosion characterized by increased neutrophil infiltration into the intestinal tissue, massive necrosis of mucosal and sub-mucosal layers, vascular dilation, oedema and sub-mucosal erosion that are noteworthy features of human erosive colitis [12]. The characteristic feature is an imbalance between oxidant and antioxidant substances [13].

Acetic acid induced erosive colitis in the colon of wistar rats bears close resemblance to human erosive colitis in terms of pathogenesis, histopathological features and

inflammatory mediator profile and is therefore a reliable animal model that can be useful for evaluation of drugs for erosive colitis[14].
Extract of *garcinia kola* has been reported to have myorelaxant effect in addition to anti-inflammatory action on animal models of erosive colitis [15].

1.2 STUDY LOCATION
The study was conducted at Histopathology Department of Faculty of Medical Laboratory Sciences, UsmanuDanfodiyo University, Sokoto, Sokoto State

1.3 ETHICAL CONSIDERATION
Ethical approval for this research was sought from the Ethics Committee on Research and Experiment of UsmanuDanfodiyo University, Sokoto.

1.4 EXPERIMENTAL ANIMALS
Adult Male wistar rats (155-204g) were procured from the animal house of the Faculty of Pharmaceutical sciences, UsmanuDanfodiyo University, Sokoto. They were kept in a well-ventilated room with optimum environmental conditions of temperature, relative humidity, dark/light cycle and were fed standard feed pellets and tap water ad libitum. They were acclimatized for two weeks prior to the experiment.

1.5 PLANT COLLECTION
Seeds of *Garcinia kola* were purchased from Sokoto Central Market (Shagari Gate) Sokoto, Nigeria. The plant material was subjected for identification and authentication at the Department of Botany, UsmanuDanfodiyo University, where a voucher number was allocated a voucher number –PCG/UDUS/GUH/0001 and deposited at the herbarium.

1.6 EXTRACT PREPARATION

The seed were cleaned and air-dried at room temperature for 7 days and ground to fine powder using mortar and pestle. Five hundred (500) grams of the powdered material was macerated in 1.5 L of distilled water and left for 24 hours after which it was filtered using Whitman's filter paper. The filtrate was dried in a hot air oven at 40^{o}C to give 39.8g of the aqueous seed extract which was used for the study. The percentage yield was calculated to be 7.96% and the dried extract stored in an airtight container [16].

1.7 ACUTE TOXICITY TESTING
Acute toxicity testing was conducted using[17]. In Phase I, nine rats were used and randomly assigned into 3 groups of 3 rats each. The 1st group was administered 10mg/kg body weight of the extract using an oral cannula, the 2nd and 3rd groups received 100mg/kg and 1000mg/kg body weight, respectively. The animals were then observed for 24 hours to monitor their behavior for signs of toxicity as well as mortality. In Phase II, three rats were used and randomly placed into 3 groups of an animal each. The animals were administered high doses of 1600mg/kg, 2900mg/kg and 5000mg/kg, respectively. They were then observed for 24 hours for signs of toxicity and mortality.

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1.8 COLITIS INDUCTION
All animals (except group I) were fasted for 6 hours prior to study, water ad libitum and given mild anesthesia before induction of erosive colitis and 2ml acetic acid (4% v/v) in 0.9% saline were infused for 30s using a soft flexible pediatric catheter size of 6F 2mm in diameter, inserted through the rectum into the colon up to a distance of 8cm and

maintained in a supine Trendelenburg position for 30 seconds to prevent leakage of the intracolonic instill [18].

1.9 EXPERIMENTAL DESIGN

Table 1: Summary of Experimental Design

Experimental group	Induction of EC	Treatment given	Duration of treatment
1 (negative control) (5 rats)	Distilled water	No treatment given	14 days
2 (positive control) (5 rats)	2mls(4% acetic acid)	No treatment given	14 days
3 (treatment group 1) (5 rats)	2mls(4% acetic acid)	100mg/kg(A.S.E.G.K)	14 days
4 (treatment group 2) (5 rats)	2mls(4% acetic acid)	200mg/kg(A.S.E.G.K)	14 days
5 (treatment group 3) (5 rats)	2mls(4% acetic acid)	400mg/kg(A.S.E.G.K)	14 days
6 (prednisolne group 4) (5 rats)	2mls(4% acetic acid)	2mg/kg	14 days

Comment [DP8]: The route of administration and duration need to be mentioned. The maintenance of the animals during the 14 days period to be mentioned.

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1.10 SCORING AND ASSESSMENT

1.10.1 Scoring Based On Stool Consistency

Every morning, stool from all rats from the six groups was examined physically and thenScored. The following scoring pattern of [19]was used

Table 2:Scoring Based on Stool Consistency

Score	Stool Consistency
0	Normal stool
1	Soft, stool but still formed
2	Soft, wet stool but unformed
3	Soft, wet stool + blood
4	Bloody diarrhea

1.10.2 Scoring Based on Macroscopic Characteristics

Pieces of rat colon (10 cm long each) were scored for macroscopic features using scoring pattern as shown in table below as described by [19]

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Table 3.Scoring Based on Macroscopic Characteristics

Score	Macroscopic changes
0	No macroscopic change

1	Mucosal erythema, hyperaemia at the sites
2	Mild mucosal oedema, slight bleeding or small erosions
3	Moderate mucosal oedema, slight bleeding erosions
4	Severe erosion, oedema and tissue necrosis

3.10.3 Scoring of Ulcer Area

The erosion area was determined by the method described by [20].

Comment [DP11]: Is it macroscopic or microscopic. A common man should be in a position to follow this article.

Table 4: Scoring of Ulcer Area

Score	Changes
0	Normal coloured colon
0.5	Red colouration
1	Spot erosion
1.5	Haemorrhagic streaks
2	Erosion ≥ 3 but ≤ 5
3	Erosion >5

Table .5: Histological Scoring Pattern.

Score	Histological Changes
0	No abnormality detected
1 (mild)	Damage / active changes up to 25%
2 moderate)	Damage / active changes more than 25% but less than 50%
3 (severe)	Damage / active changes of more than 50%

Using the method of[21], the histomorphological parameters analyzed were erosive cell infiltrate (for severity and extent), epithelial changes (to assess hyperplasia and erosion) and mucosal architecture (for goblet cell loss and altered crypts).

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1.11 DATA AND STATISTICAL ANALYSIS

All the results were expressed as mean ± S.D. Data analysis was performed using GraphPad Prism 6.0 software (GraphPad, San Diego, USA). Statistical comparison between drug-treated groups and colitis control animals was done using one-way ANOVA. A value of $p < 0.05$ was considered to be statistically significant.

1.12 RESULTS

Table .6: The Physical Properties of Aqueous seed Extract of *Garcinia kola*

Plant Part	Extract Type	% Yield	Texture	Colour	Smell
Seed	Aqueous	7.96%	Soft gel	Brown	Sweet

Table .7: Acute Toxicity of aqueous seed extract of *Garcinia kola* Wistar Rats (N=12)

Dose	Mortality	
	Phase I	Phase II
10mg	0/3	-
100mg	0/3	-
1000mg	0/3	-
1600mg	-	0/1
2900mg	-	0/1
5000mg	-	0/1

Table .9: Effect of A.S.E.G.K. on some physical parameters.

Parameters	Normal	Acetic acid	*Garcina kola*			P-Values
		Control	100mg/kg	200mg/kg	400mg/kg	2mg/kg	
SC	1.00 ± 0.00	3.00 ± 0.00	1.50 ± 0.29	1.25 ± 0.25	1.25 ± 0.25	1.25 ± 0.25	0.000
MIC	0.00 ± 0.00	3.75 ± 0.25	2.75 ± 0.25	2.25 ± 0.25	1.25 ± 0.25	0.25 ± 0.25	0.000
MAC	0.00 ± 0.00	3.75 ± 0.25	2.75 ± 0.25	2.25 ± 0.25	1.25 ± 0.25	0.25 ± 0.25	0.000

Keynote: Data are expressed as Mean±SD. SC- Stool consistency, MIC- Microscopic characteristic and MAC- Macroscopic characteristics

Table .10: Effect of A.S.E.G.K. on Macroscopic Colonic Features.

Parameters	Normal	Acetic acid	*G. kola*			P-Values
		Control	100mg/kg	200mg/kg	400mg/kg	2mg/kg	
MAC	0.00 ± 0.00	3.75 ± 0.25	2.75 ± 0.25	2.25 ± 0.25	1.25 ± 0.25	0.25 ± 0.25	0.000

Keynote: Data are expressed as Mean±SD.

Table 11: Effect of A.S.E.G.K. on Colon Histological Parameters

Parameter	normal	Acetic acid	*G.kola*			Prednisolone	P-Values
		Control	100mg/kg	200mg/kg	400mg/kg	2mg/kg	

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Comment [DP14]: I presume that the physical properties of stool in various groups are described and the mean frequency with standard deviation is described. I could not understand this 0.00.

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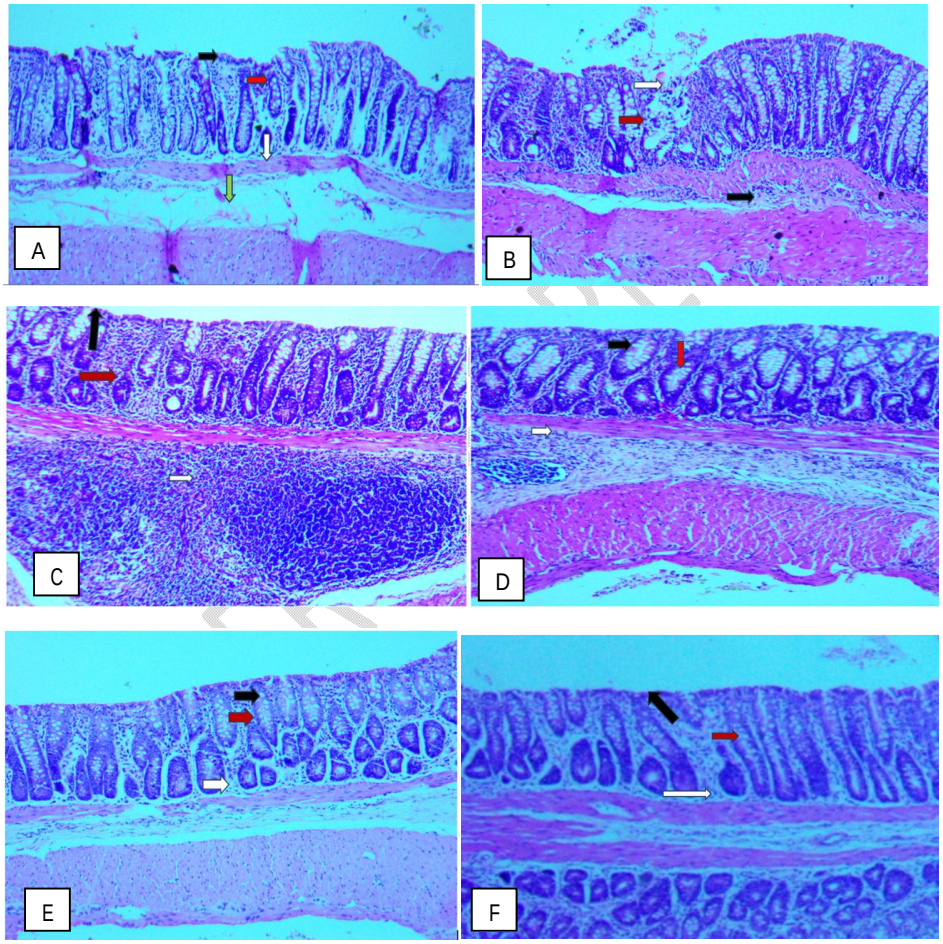
Infiltrates	0.00 ± 0.00	3.75 ± 0.259	3.25 ± 0.25	2.25 ± 0.25	1.75 ± 0.48	0.75 ± 0.25	0.000
Severity							
Epithelial Hyperplasi	0.25 ± 0.10	3.75 ± 0.25	3.00 ± 0.00	2.25 ± 0.29	1.50 ± 0.29	0.50 ± 0.29	0.000
Goblet cell loss	0.00 ± 0.00	3.75 ± 0.25	3.25 ± 0.25	2.50 ± 0.25	1.50 ± 0.29	0.75 ± 0.25	0.000
Crypts alteration	0.00 ± 0.00	3.75 ± 0.25	3.25 ± 0.25	2.25 ± 0.25	1.50 ± 0.29	0.75 ± 0.25	0.000

Keynote: Data are expressed as Mean±SD.



PlateA: Photomicrograph of Colonic Tissue from Control Animal. Intact colonic mucosa from control Animals showing normal crypts (black arrow), goblet cells (red arrow), sub mucosa (white arrow), and muscularis propria (green arrow) (H&E. Mag x 100)

Plate B: Photomicrograph of Colonic Tissue from Colitis Control Group. Section shows oedema (black arrow), numerous inflammatory cells extending to the submucosa (black arrow), distortion of crypts and goblet cells and mucosal erosion (red arrow), and mucosal erosion (white arrow) (H&E. Mag x 100).

Plate C: Photomicrograph of Colonic Tissue from Animal Receiving 100mg/kg of the Extract. Section shows mild crypt distortion (black arrow) with a few goblet cells (red arrow). Inflammatory cells are seen in the mucosa and submucosa (white arrow) (H&E. Mag x 100)

Plate D: Photomicrograph of Animals Receiving 200mg/kg of the Extract Section shows almost normal crypts (black arrow) with normal goblet cell numbers (red arrow). Infiltrates cells are seen mostly within the mucosa and submucosa (white arrow) (H&E. Mag x 100)

Plate E: Photomicrograph of Animal Receiving 400mg/kg of the Extract. Section shows normal goblet cells (black arrow) and normal crypts (red arrow). A clearing of inflammatory cells and polymorphs are seen in the mucosa (white arrow). (H&E. Mag x 100)

Plate F: Photomicrograph of Colonic Tissue from Treatment Control Group. Section shows normal crypt (black arrow) with adequate amount of goblet cells (red arrow). Polymorphs are scanty (white arrow). There is a clearing of most infiltrates by Prednisolone. (H&E. Mag x 100)

1.13 DISCUSSION

The extraction yield is a measure of the solvent efficiency to extract specific components from an original material. The aqueous extraction method used in this research produced a percentage yield of 7.96%. This is in agreement with the work of [22]. The extract was brown in colour, a crystalline shine and a slight sweet smell. [23]. Research by [22] has shown that several compounds are absent or present in different quantities from plants from different provenances, indicating the presence of chemotypes. This may account for the similar results in the percentage yield of the crude extract.
In the acute toxicity study, all the graded doses up to 5000mg/kg showed no sign of toxicity in the animal and no mortality was recorded in the study. The LD50 of aqueous seed extract of *Garcinia kola* was found to be higher than 5000mg/kg body weight. This result is consistent with the findings of [24], and [25][26], [27], [28], [29].

Treatment of erosive colitis rats with the aqueous seed extract of *Garcinia kola* in this study reduced erosion score. *Garcinia kola* contains biflavonoids and bioflavonoids have been shown to stimulate angiogenesis, an important facet of tissue healing [30].
It was observed that animals receiving the aqueous seed extract of *Garcinia kola* showed improvement in stool consistency in a dose dependent manner. It was also seen in animals receiving the extract but improved remarkably in a dose dependent manner. Animals receiving the highest dose of the extract showed comparatively similar results to normal control groups. It was also observed that *Garcinia kola* extract ameliorated diarrhoea score[31]. Swelling is one of the symptoms of inflammation. The result of the study indicated that treatment of erosive colitis rats with aqueous seed extract of *Garcinia kola* decreased tissue thickness. Histopathological damage was evaluated in colonic samples stained with haematoxylin and eosin. In the normal animals, epithelial crypts of the mucosal layer were intact. There was no infiltration of inflammatory cells. The intra-rectal instillation of acetic acid resulted in significant development of transmural necrosis, submucosal oedema, erosion along with cellular infiltration and loss of epithelial crypts and goblet cells. Animals fed with the extract showed reduction in the extent of damage in a dose dependent manner) 100mg/kg, 200mg/kg and animals receiving the control drug - Prednisolone (2mg/kg). Animal fed with the highest dose 400mg/kg showed clearing of

inflammatory cells with decreased goblet cells.This result is consistent with the findings of Fiot*et al*.[24], and [25,26. 27, 28, 29].

The histological studies of erosive colitis treated rats reveals regenerating mucosal layer with regenerating colonic crypts and goblet cells (figure 1 and figure 2) in contrast to the histology of colitis rats which are characterized by indistinguishable colonic layers (plate 2). This result is consistent with the findings of [24], and [25, 26, 27. 28, 29].

Microscopically, mucosal and sub-mucosal inflammatory cellular infiltrations were detected. It was noted that the animals that received acetic acid showed a significant increase in mucosal and submucosal inflammatory cellular infiltrates compared to the control animals. It also indicated that there was a mild decrease in the severity but a marked improvement in the extent of mucosal and submucosal inflammatory cellular infiltration in animals receiving the extract. 400mg/kg treatment group cleared all polymorphs in the colon when compared to Prednisolone which is a standard drug of choice. This is indicative of the superiority and relevance of action of high dose of *garcinia kola* on the inflammatory cells. More so, Prednisolone which is a standard drug of choice effectively reduced all histological changes such as oedema, crypt distortion, and goblet cell loss and tissue injury by virtue of its healing property which is in accordance with, [16] and [32]

1.14 CONCLUSIONS

The present study showed that treatment of colitis rats with *Garcinia kola* extract decreased erosion score by stimulating colonic healing and reduced diarrhea score and tissue thickness. The extract also improved weight of erosive colitis rats in 14 days after treatment. Therefore, aqueous seed extract of *Garcinia kola* exhibited therapeutical effect. Oral administration of aqueous extract of the seed of *Garcinia kola* at doses used in this study showed notable improvements in body weight compared to erosive colitis control animals.

Though, the effect of Prednisolone which is a standard drug of choice was not as potent as to 400mg/kg treatment group used in the study. Prednisolone which is a standard drug of choice also showed remarkable improvement in the scores of both macroscopic and microscopic colonic parameters compared to control groups.

In conclusion, results of this study provide scientific evidence which lend credence to the use of *Garcinia kola* extract in the folklore medicine in the management of colitis erosion.

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Comment [DP19]: No data given.

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Comment [DP22]: Is it relevant?