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Beneficial Effects of Guiera Senegalensis on Selected Parameters in Acetic Acid-Induced Colitis in Wister Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Inflammatory bowel disease (IBD) comprises Crohn's disease (CD) and ulcerative colitis (UC) and are defined as chronic and relapsing inflammations of the gastrointestinal tract caused by variable pathophysiological mechanisms. Synthetic chemical moieties with antioxidant potential are the present treatment regimens, but their high relapse rate and toxicities limit their utility in treatment.

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Aim: The aim of this work is to investigate the possible beneficial effects that the aqueous extract of the plant will elicit on selected parameters in experimental rats. A control, colitis control and a treatment control will serve as a guide in the assessment of the findings in this study.
Methods: Experimental colitis was induced in animals using acetic acid to mimic human IBD. An aqueous extraction method of the plant was used to reflect traditional uses. The effects of oral administration of the extract in the animals were compared using a control, colitis control and treatment control (Prednisolone).
Results: There were statistically significant and dose-dependent improvements in food intake, stool consistency, body weight and microscopic colonic changes of test animals compared to control groups but not as remarkable as the treatment control. The extract also showed remarkable improvement in the scores of both macroscopic and microscopic colonic parameters compared to control groups. Also, the findings were not as potent as prednisolone. Water intake and splenic weight, on the other hand, were better in animals receiving the extract compared to those receiving prednisolone. The extract, however, does not bear the side effects of immune suppression and toxicity that prednisolone has as evidenced by splenic weights measured.
Conclusion: The extract is safe for consumption and has shown anti-inflammatory and healing properties. Prednisolone (2mg/kg) showed slightly better anti-inflammatory properties than the extract at doses used in this study (≤400mg/kg). The extract, however, doesn't seem to have the side effects of prednisolone.

Keywords: Guiera senegalensis; acetic acid; colitis; anti-inflammatory.

1. INTRODUCTION

Inflammatory bowel disease (IBD) comprises Crohn's disease (CD) and ulcerative colitis (UC) which are defined as chronic and relapsing inflammations of the gastrointestinal tract caused by variable pathophysiological mechanisms characterized by clinical manifestations including diarrhoea, blood in the stool, abdominal pain, and weight loss [1]. Despite the fact that aetiology of IBD still remains poorly understood, complex interactions among genetic, environmental, immunological and reactive oxygen species (ROS) have been implicated in the pathogenesis of IBD [2,3]. IBD occurs throughout the world but is more common in urban areas and presents in the teens and early 20s [4].

Although there are few epidemiologic data from developing countries, epidemiological studies from all over the world have stated that the incidence and prevalence of IBD are increasing with time and in different regions around the world — indicating its emergence as a global disease [5].

Guiera senegalensis (Family: Combretaceae) commonly known as 'Sabara' in Hausa is a shrub of the savannah region of West and Central Africa [6]. Elemental analysis showed that *G. senegalensis* leaves contain a significant amount of essential mineral elements. This justifies the widespread usage of *G. senegalensis* leaves as medicine traditionally [7]. Several studies have indicated the presence of alkaloids**,** flavonoids, quercetin, catechins, saponin, tannins, amino acids, ascorbic acid, anthraquinones and a bitter principle, elastine, in the roots and leaves of *G. senegalensis* with potential anticancer and other forms of biological activities [8]. Their functions and mechanism of actions may include the following among others: antioxidant activity, hormonal action, stimulation of enzymes, interference with DNA replication and antibacterial properties [9]. Other plant extracts that have shown promise in the management of this condition includes cannabigerol (CBG), a non-psychotropic Cannabis-derived cannabinoid [10], CBD-BDS, a cannabidiol [11] and Palmitoylethanolamide, an acylethanolamide related to a cannabinoid, anandamide [12]

WHO estimates that of the 35,000 - 70,000 species of plants that are used for medicinal purposes around the world, only about 5,000 have been submitted for biomedical scrutiny [13]. In the Northern part of Nigeria, the leaves are used for the traditional treatment of dysentery, diarrhoea, pile and stomach ache [14], breast cancer and associated breast inflammatory lesions (e.g. mastitis) [15]. In Nigeria, the recommended cultural practices for treatment include regular drinking and taking bath with fresh water decoction of the leaves [15].

Synthetic chemical moieties like 5-amino salicylate, corticosteroids, antimicrobials and

immunosuppressive agents such as azathioprine and mercaptopurine, etc. with antioxidant potential are the present treatment regimens for IBD. But, their disadvantages like high relapse rate, immune suppression and a wide range of side effects limit their utility in the treatment of IBD [16]. Acetic acid-induced colitis bears close resemblance to human IBD in terms of pathogenesis, histopathological features and inflammatory mediator profile [17] and is, therefore, a reliable animal model that can be useful for evaluation of drugs for IBD [18]

The aim of this work is to investigate the histopathological effects that the extract of the plant will elicit on parameters of experimental animals. A control, colitis control and a treatment control will serve as a guide in the assessment of the findings in this study.

2. METHODOLOGY

2.1 Experimental Animals

Adult Wistar rats (110-150g) were procured from the animal house of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo 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 commencement of the experiment.

2.2 Plant Collection

The fresh leaves of *G. senegalensis* used for this study were collected from a bush around Arkilla, Wammako Local Government Area of Sokoto State, Nigeria. The plant was authenticated by the Herbarium Officer at the Botany unit of Usmanu Danfodiyo University, Sokoto. It was given a voucher number – UDUH/ANS/0144 and deposited at the herbarium.

2.3 Extract Preparation [19]

The leaves were cleaned and air-dried at room temperature for 7 days and ground to fine powder using mortar and pestle. Three hundred and fifty (350) 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 Whatman's filter paper. The filtrate was dried in a hot air oven at 40^{o}C to give 34.5g of the aqueous leaf extract which was used for the study. The percentage yield was calculated to be 9.86% and the dried extract stored in an airtight container.

2.4 Acute Toxicity Testing

Acute toxicity testing was conducted using Lorke's Method [20]. In Phase I, nine (9) 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 1000 mg/kg body weight respectively. The animals were then observed for 24 hours to monitor their behaviour for signs of toxicity as well as mortality. In Phase II, three (3) rats were used and randomly placed into 3 groups of an animal each. The animals were administered high doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively. They were then observed for 24 hours for signs of toxicity, morbidity and/or mortality.

2.5 Colitis Induction

All animals (except group I) were fasted for 6 hours prior to study, with access to water *ad libitum* and given mild anaesthesia before induction of colitis, 2ml acetic acid (4% v/v) in 0.9% saline were infused for 30s using a soft flexible paediatric catheter size of 6F 2 mm in diameter, inserted through 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 instil [21]

2.6 Experimental Design

The research design employed a slight modification of the method of Jagtap *et al.* [22]. The study comprised of thirty (30) animals. The animals were divided into six groups (I - VI). Each group consists of five (5) animals each. Food intake, water intake, stool consistency and body weights of the animals were monitored daily.

On the 8th day, final body weight was measured and animals were sacrificed by anaesthetic overdose. Colons and spleen were removed, excised and rinsed with normal saline. Necessary measurements were made and oriented using the swiss roll technique [23]. They were then transferred to 10% formal saline for fixation. Tissues were processed using paraffin embedding techniques and stained with H&E.

Table 1. Experimental design

Group	Saline (intrarectally)	4% Acetic acid (intrarectally)	A.L.E.G.S. (orally)	Prednisolone (orally)
I (control)	2ml Day 1	-	-	-
II (Colitis control)	-	2ml Day 1	-	-
III	-	2ml Day 1	100mg/kg Day 1-7	-
IV	-	2ml Day 1	200mg/kg Day 1-7	-
V	-	2ml Day 1	400mg/kg Day 1-7	-
VI (treatment control)	-	2ml Day 1	-	2mg/kg Day 1-7

Note: A.L.E.G.S. stands for Aqueous Leaf Extract of Guiera senegalensis

2.7 Scoring and Assessment

Stool consistency was assessed using scoring pattern of Masonobu et al. [24] as follows

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

Pieces of rat colon (10 cm long each) were scored for macroscopic features using the scoring pattern shown below as per the method of Deshmukh et al. [25]

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 ulcer or erosions
4	Severe ulceration, oedema and tissue necrosis

The ulcer area was determined as per the method described by Dengiz and Gursan [26]

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

Histological scoring was graded using the method of Patil et al. [27]

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%

Referring to the guidelines of Erben et al. [28], the histomorphological parameters analysed were inflammatory cell infiltrate (for severity and extent), epithelial changes (to assess hyperplasia and erosion) and mucosal architecture (for goblet cell loss and altered crypts).

Results are presented as Mean±SD. Data analysis was performed using Graph Pad Prism 7.0 software (GraphPad, San Diego, USA). Statistical comparison between drug-treated groups and colitis control animals was done using one-way ANOVA and the Kruskal-Wallis Test where applicable. A value of $p < 0.05$ was considered to be statistically significant.

3. RESULTS

The results of this experiment are grouped based on the category of parameters – macroscopic and microscopic, parametric and non-parametric etc. This is done to ease the choice of statistical tool in the analysis of the result. Consequently, each facet of the condition can be assessed individually rather than generalized.

3.1 Effect of A.L.E.G.S. on Food Intake and Water Intake

The food and water intake of each group were monitored daily. The food intake of animals which received the extract was increased in a dose-dependent manner. However, their food intake was not as high as the normal control.

Animals in the normal control group drank the most water. The acetic acid group drank significantly less water and the intake increased in a dose-dependent manner in animals receiving the extract. Animals taking prednisolone consumed the least amount of water during the study.

3.2 Effect of A.L.E.G.S. on Some Physical Parameters

The percent decrease in body weight was calculated daily and the stool consistency was graded and recorded daily. Animals receiving the extract showed improvement in weight loss and stool consistency in a dose-dependent manner. Colitis control animals showed splenomegaly. 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.

3.3 Effect of A.L.E.G.S. on Macroscopic Colonic Features

After intra-rectal instillation of 4% acetic acid, the colons were weighed and examined for signs of haemorrhage and ulceration. Animals receiving the extract showed improvement in colon weight as compared to the colitis control groups. The mean microscopic score in acetic acid control rats was significantly increased ($P < 0.01$) as compared to normal rats. The 7 days' treatment with the extract significantly and dose-dependently decreased these macroscopic lesions produced by the intra-rectal instillation of acetic acid.

3.4 Effect of A.L.E.G.S. on Histological Changes of Rat Colon

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, ulceration along with cellular infiltration and loss of epithelial crypts and goblet cells.

Animals fed with the extract showed a reduction in the extent of damage in a dose-dependent manner. Animals receiving the control drug - prednisolone (2mg/kg) showed clearing of inflammatory cells with decreased goblet cells.

4. DISCUSSION

Acute toxicity study revealed no morbidity, behavioural changes or mortality in the rats indicating that the lethal dose is above 5000mg/kg. This is an indication that the extract is safe for consumption. This result is consistent with the findings of several authors including [6-9,15]. The present investigation demonstrated that acetic acid-induced colitis was associated with macroscopic and microscopic changes. The intrarectal instillation of acetic acid resulted in a massive localized erosion of the colonic mucosa leading to severe localized inflammation and haemorrhage. This was established by numerous authors [17,18,29]

Loss of appetite is one of the symptoms of IBD and is measured by reduction in food intake. This is due to the irritation of the gastrointestinal tract and the inflammatory processes that characterize the disease [1]. Acetic acid-induced colitis produced similar effect [27]. The colitis control had a significantly low mean food intake (50.25g/group/day) compared to normal control (110.5 g/group/day). Animals receiving the extract showed improvement in food intake in a dose-dependent manner. Animals administered with prednisolone, however, showed more improvement than those receiving the highest dose of the extract. Nonetheless, the colitis group began to show improvement on their own at about the 5th day of the experiment. Fabia et al. [21] supported this finding as well as asserting that symptoms begin to heal on their own at about 4 days after colitis induction.

Water intake can also serve as a marker of GIT irritation as well as a marker for assessing the level of dehydration. As compared to normal rats, water intake in the acetic acid control rats was significantly decreased. Administration of the extract for 7 days significantly and dose-dependently increased the water intake of rats.

Table 2. Effect of A.L.E.G.S. on food intake and water intake

Parameter	Normal	Acetic acid control	*Guiera senegalensis*			Prednisolone 2mg/kg
			100mg/kg	200mg/kg	400mg/kg	
Food intake (g)	110.50±9.37	50.25±8.68	61.50±3.87	69.75±5.97	78.25±8.88	88.00±9.31
Water intake (ml)	293.10± 23.24	220.75±29.53	255.25±25.53	278.75±25.78	282.50±29.85	200.0±24.81

Data are presented as Mean±SD. One-way ANOVA displayed an F value of 0.04454, R square value of 0.03441 and a p-value of 0.9950. The difference among means is considered not statistically different. Brown-Forsythe test displayed an F (DFn, DFd) value of +infinity (4, 5) and a p-value of <0.0001. There is a statistically significant difference between the SDs.

Table 3. Effect of A.L.E.G.S. on some physical parameters

Parameter	Normal	Acetic acid control	*Guiera senegalensis*			Prednisolone* 2mg/kg
			100mg/kg	200mg/kg	400mg/kg	
% decrease in body weight	-2.25±1.22	5.02±2.90	1.29±6.24	0.71±2.40	-1.07±5.79	-1.07±5.44
Stool Consistency	0.00±0.00	1.50±0.76	1.33±0.75	1.23±1.07	1.03±1.49	0.67±0.75
Spleen Weight (g)	0.53±0.13	0.89±0.24	1.05±0.08	0.56±0.35	0.52±0.07	0.70±0.11

*Data are expressed as Mean±SD. A Kruskal-Wallis H test showed that there is a statistically significant difference between the different groups. P = 0.0303. Kruskal-Wallis statistic = 8.958; * Has the lowest mean score and therefore ranked highest according to Kruskal-Wallis Test*

Table 4. Effect of A.L.E.G.S. on macroscopic colonic features

Parameter	Normal	Acetic acid control	*Guiera senegalensis*			Prednisolone* 2mg/kg
			100mg/kg	200mg/kg	400mg/kg	
Colon Weight (g)	1.50±0.07	1.62±0.11	1.56±0.33	1.55±0.26	1.52±0.23	1.47±0.06
Macroscopic score	0.00±0.00	2.50±0.45	2.25±0.39	1.33±0.41	1.25±0.39	1.00±0.00
Ulcer Score	0.00±0.00	2.33±0.41	1.83±0.20	1.33±0.20	1.0±0.31	0.67±0.20

Data are expressed as Mean±SD. A Kruskal-Wallis H test showed that there is a statistically significant difference between the different groups. P = 0.0026. Kruskal-Wallis statistic = 11.13; Has the lowest mean score and therefore ranked highest according to Kruskal-Wallis Test*

Table 5. Effect of A.L.E.G.S. on colon histological parameters

Parameter	Normal	Acetic acid control	*Guiera senegalensis*			Prednisolone* 2mg/kg
			100mg/kg	200mg/kg	400mg/kg	
Infiltrates Severity	0.25±0.43	1.75±0.43	1.50±0.50	1.00±0.00	0.75±0.43	0.00±0.00
Infiltrates Extent	0.25±0.43	1.50±0.50	1.25±0.43	1.25±0.43	1.00±0.00	0.00±0.00
Epithelial Hyperplasia	0.00±0.00	1.50±0.50	1.00±0.00	0.50±0.50	0.25±0.43	0.50±0.50
Epithelial Erosion	0.00±0.00	0.50±0.50	0.25±0.43	0.00±0.00	0.00±0.00	0.00±0.00
Goblet cell loss	0.00±0.00	2.00±0.70	0.75±042	0.25±0.43	0.00±0.00	1.25±0.43
Crypts alteration	0.00±0.00	2.25±0.43	1.25±0.43	0.25±0.43	0.25±0.43	0.25±0.43

*Data are expressed as Mean±SD. A Kruskal-Wallis H test showed that there is a statistically significant difference between the different groups. P = 0.0054. Kruskal-Wallis statistic = 14.67; * Has the lowest mean score and therefore ranked highest according to Kruskal-Wallis Test*

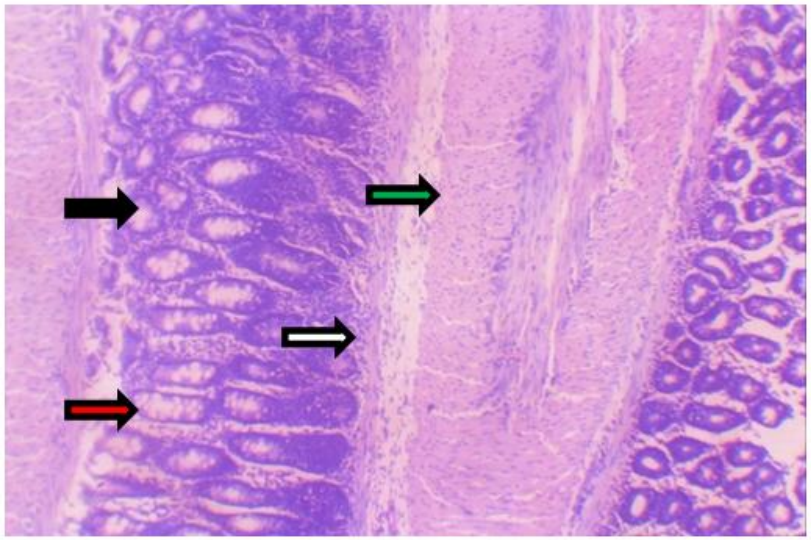

Plate 1. Photomicrograph of colonic tissue from a control animal. Intact colonic mucosa from control animals showing normal crypts (black arrow), goblet cells (red arrow), submucosa (white arrow) and muscularis propria (green arrow). (H&E. Mag. x 100)

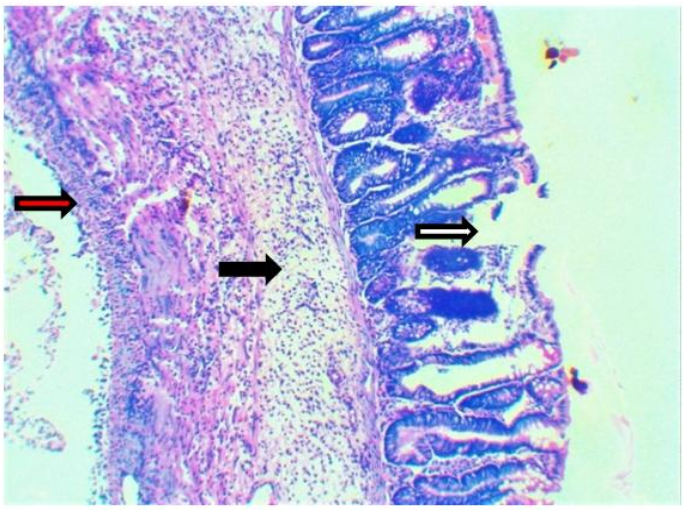

Plate 2. Photomicrograph of colonic tissue from colitis control group. The section shows oedema (black arrow). There are numerous inflammatory cells extending up to the muscularis propria (red arrow) with mucosal erosion (white arrow). (H&E. Mag. x100)

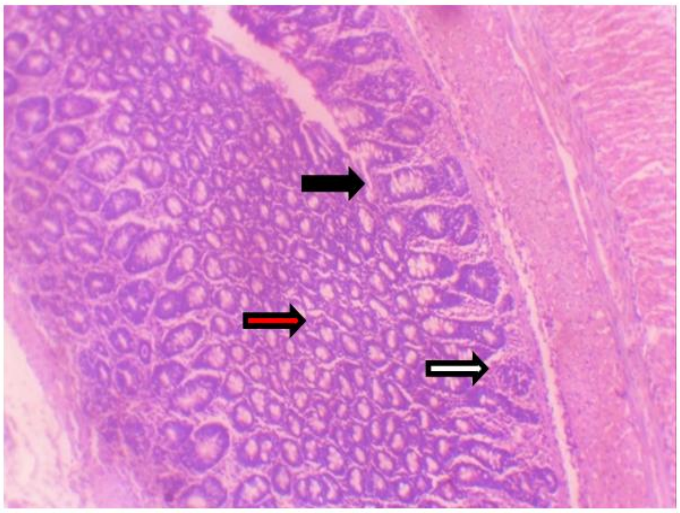

Plate 3. Photomicrograph of colonic tissue from animal receiving 400 mg/kg of the extract. The section shows normal goblet cells (black arrow) and near normal crypts (red arrow). No inflammatory cells are seen in the mucosa (white arrow). (H&E. Mag. x100)

Improvement in the water intake was better in those receiving the extract compared to those receiving prednisolone. Sewell et al. [30] supported the finding that prednisolone reduces water intake in rats.

Due to variations in animal weight, the best marker for assessing weight loss is percentage decrease in body weight [1,27]. An abundant literature has asserted that acetic acid-induced colitis is characterized by weight loss. Consequently, weight loss is a feature of IBD [31]. This experiment shows a dose-dependent improvement in the weight of the animals during the study with no recorded weight loss in animals receiving 400 mg/kg of the extract and those receiving prednisolone. Prednisolone has also been shown to aid in weight gain [30].

Stool consistency as a marker of diarrhoea can be used to assess severity and extent of disease [17]. Colitis control animals showed marked diarrhoea with mucus and blood. Oral administration of the extract caused

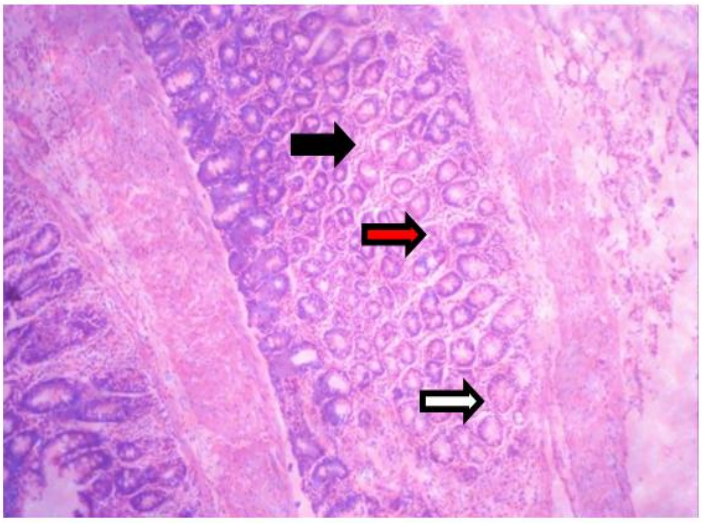

Plate 4. Photomicrograph of colonic tissue from treatment control animal. The section shows mild crypt changes (black arrow) with a reduced amount of goblet cells (red arrow). Lymphocytes are absent in the sections (white arrow). There is a clearing of the infiltrates by prednisolone. (H&E. Mag. x100)

a reduction in these symptoms dose-dependently as well as those receiving prednisolone. This supported the findings of Shettima et al. [32] using a methanolic extract and Jigam et al. [8] who in addition noted that the extract has anti-inflammatory properties.

The spleen is considered the draining site for compounds that are administered into the system and is therefore considered an essential part of the immune system and reticuloendothelial system [33] as well as an important organ to evaluate for treatment-related lesions [33,34]. Acetic acid-induced colitis was associated with splenic enlargement. The extract used in this study significantly decreased the splenic enlargement probably via its immunomodulatory potential. Animals receiving higher doses of the extract had a better mean splenic weight (0.56 and 0.52 g respectively) than those receiving prednisolone (0.71 g). This shows that the extract does not have the immunotoxic effects of prednisolone [35].

Increased weight of colon is reflecting the degree of local inflammation along with the other parameters of oedema and wall thickening. The weight of the colon tissue is elevated due to an inflammatory response which is indicative of severity and extent of the disease [27,36-38]. There was decrease in the wet weight of colonic tissue in animals receiving the extract in a dose-dependent way, though; those animals receiving prednisolone recorded better colonic tissue weights.

The macroscopic score is an indication of gross lesions caused by intrarectal instillation of 4% acetic acid. A higher score indicates increasing severity of disease [27]. Noteworthy features of the colitis include erythema, oedema, bleeding, ulceration and necrosis. Administration of the extract showed improvement in the grade of damage as well as tissue healing. The score was decreased as the concentration of the extract increased. Animals receiving prednisolone showed a better score than those receiving the extract.

Ulcer area is reflecting the degree of the gross morphological lesions as well as necrotic area of various sizes [27]. Ulcer area was quantitatively graded and was significantly decreased in animals receiving the extract compared to colitis control animals. The effect exerted was in a dose-dependent manner. This might depict its microflora modulatory effect on the corrosive effect of acetic acid. This assertion is supported by several researchers [39-41].

Microscopically, mucosal and submucosal inflammatory cellular infiltration was detected. It was noted that the animals that the colitis group

showed a significant increase in mucosal and submucosal inflammatory cellular infiltrate compared to the control animals. It was also noted that there was a marked improvement in the extent of mucosal and submucosal inflammatory cellular infiltration in animals receiving the extract. Prednisolone, on the other hand, cleared all polymorphs in the colon of treatment control animals. This is indicative of the superior action of prednisolone on the inflammatory cells [1-3] compared to the doses of the extract administered.

Furthermore, the extract effectively reduced some histological changes such as oedema, crypt distortion, goblet cell loss and tissue injury by virtue of its healing property. A number of authors have acknowledged the healing property of the extracts of this plant [19,42,43].

The exact mechanism of anti-inflammatory and healing effect of the extract has not been clarified in this study. Several researchers [8,19,44] have shown that Guiera senegalensis may have an anti-inflammatory activity. Other authors have shown that it has antimicrobial and microflora modulatory effects [39-41]. The presence of adequate goblet cells in the mucosa of rats receiving the extract shows an increase in absorptive functions and bowel movement. This may explain the improvement in IBD symptoms.

5. CONCLUSION

The WHO promotes facilitating the integration of traditional and complementary medicine into policies of national health system by the promotion of its evidence-based use. It also encourages strategic research into alternative medicines by boosting clinical research projects on its safety and effectiveness [45].

Oral administration of aqueous extract of the leaves of Guiera senegalensis at doses used in this study showed notable improvements in parameters such as food intake, water intake, body weight and stool consistency compared to colitis control animals. Though, the effect was not as potent as the control treatment drug (prednisolone) used in the study.

The extract also showed remarkable improvement in the scores of both macroscopic and microscopic colonic parameters compared to control groups. Also, the findings were not as potent as prednisolone. The extract, however, does not bear the side effects of immune suppression and toxicity that prednisolone has as evidenced by splenic weights measured.

In addition to other researches by several authors especially on the anti-inflammatory and healing properties [8,19,46], The extract is safe for consumption and has shown anti-inflammatory and healing properties. Prednisolone (2 mg/kg) showed slightly better anti-inflammatory properties than the extract at doses used in this study (≤400 mg/kg). The extract, however, doesn't seem to have the side effects of prednisolone.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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