wjpmr, 2018,4(3), 310-315

SJIF Impact Factor: 4.639

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

EFFECTS OF GUIERA SENEGALENSIS IN THE SPLEENS OF RATS IN AN ANIMAL MODEL OF COLITIS

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Article Received on 19/01/2018 **Article Revised on 09/02/2018** **Article Accepted on 01/03/2018**

ABSTRACT

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. Synthetic chemical moieties with antioxidant potential are the present treatment regimens, but their high relapse rate and toxicities limits their utility in treatment. **Aims:** The aim of this work is to investigate the possible toxic effects that the aqueous extract of the plant will elicit on the spleen 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. **Methods:** Experimental colitis was induced in animals using acetic acid to mimic human IBD the effects of oral administration of the extract on the spleen was compared using a control, colitis control and treatment control (Prednisolone). **Results:** Colitis animals showed numerous germinal centres in the white pulp with some congestion of blood vessels and necrosis in the red pulp. Treated animals showed comparatively normal white and red pulp with a discernible increase in marginal zones. Treatment control animals showed an expansive white pulp with few germinal centres of low cellularity **Conclusion:** The extract showed no toxicity to the spleen unlike prednisolone. The histological findings reveal immunomodulatory functions.

KEYWORDS: Inflammatory bowel disease, Crohn's disease, ulcerative colitis.

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. *Guiera senegalensis* has also been shown to positively contain alkaloids, saponins, tannins, flavonoids, amino acids, ascorbic acid, and anthraquinones and also displayed antimicrobial activity. Alkaloids and saponins have received the greatest attention with regards to their possible medicinal potential.[6] Elemental analysis showed that the values of all the elements analysed compares favourably with values obtained for other plants and thus indicated that *G. senegalensis* leaf contain significant amount of essential mineral elements. Their quantity is in the order Ca > K > P > Na > Mg > Fe > Zn > Cu. This justified the widespread usage of *G. senegalensis* leaves as medicine traditionally and also showed that the plant has a lot of potentials in traditional and orthodox medicine.[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]

The spleen is an organ in the upper far left part of the abdomen and is a part of the mononuclear-phagocytic system; it removes old red blood cells, metabolizes haemoglobin, recycles iron, and holds a reserve of blood, which can be valuable in case of hemorrhagic shock.[10] The spleen synthesizes antibodies in its white pulp and removes antibody-coated bacteria and antibody-coated blood cells by way of blood and lymph node circulation.[11] A study published in 2009 using mice found that the red pulp of the spleen forms a reservoir that contains over half of the body's monocytes.[12] These monocytes, upon moving to injured tissue (such as the heart after myocardial infarction), turn into dendritic cells and macrophages while promoting tissue healing.[12]

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 wide range of side effects limits their utility in treatment of IBD.[13]

The aim of this work is to investigate the possible effects that the extract of the plant will elicit on the spleen 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.

METHODOLOGY

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 experiment.

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.

Extract Preparation[14]

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 Whatmann'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.

Acute Toxicity Testing

Acute toxicity testing was conducted using Lorke's Method.[15] 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 1000mg/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 1600mg/kg, 2900mg/kg and 5000mg/kg respectively. They were then observed for 24 hours for signs of toxicity, morbidity and/or mortality.

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 instill.[16]

Experimental Design

Colonic inflammation was induced in fasted rats using a modification of the method of Jagtap *et al.*[17] The study comprised of thirty (30) animals and were divided into six groups (I - VI). Each group consists of five (5) animals. Body weights of the animals were also monitored daily.

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.*

On the 8th day, final body weight was measured and animals were sacrificed by anaesthetic overdose. Spleen were excised, washed in normal saline and weighed. They were then transferred to 10% formal saline for fixation. Tissues were processed for 12 hours using alcohol, xylene and paraffin embedded for light microscopic study. Paraffin embedded tissue sections cut at 3µm thickness were prepared and stained after deparaffinization using Haematoxylin and Eosin (H&E) staining method to verify morphological assessment of splenic architecture.

Results are presented as Mean±SD. 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.

There isn't any statistical analysis of results or tables generated for the histological findings because it is recommended to use descriptive terms to assess splenic investigation; this is consistent with the STP position paper: Best Practice Guideline for the Routine Pathology Evaluation of the Immune System.[18]

RESULTS

Acute toxicity study revealed no morbidity, behavioural changes or mortality in the rats indicating that the lethal dose is above 5000mg/kg. There was negligible difference in the volume of bone marrow aspirated from the femurs of the experimental animals. However, microscopic changes were evident.

Control animals showed normal splenic histological features with normal white pulp containing peri-arteriolar lymphoid sheaths (PALS), lymphoid follicles and marginal zone. The red pulp was also adequate and within normal limits. Acetic acid induced colitis caused a reduction in cellularity of the white pulp and areas of necrosis in the red pulp including fibrosis.

Administration of the extract, however, caused an increase in the cellularity of the white pulp components and reduced the necrotic areas of the red pulp compared to colitis control animals. Animals receiving higher doses of the extract showed essentially normal splenic architecture with an increase in marginal zone. Animals treated with prednisolone showed plasma cells and lymphoid follicles with decreased cellularity. The red pulp also has areas of necrosis.

Table 1: Observed physical parameters.

Parameter	Normal	Acetic Acid	*Guiera senegalensis*			Prednisolone*
		Control	100mg/kg	200mg/kg	400mg/kg	2mg/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
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

One way ANOVA yielded the following – F = 0.04778; R square = 0.005333 and a P value of 0.8377 hence. Considered NOT statistically significant.

Table 2: Observed effect on various Histological splenic parameters.

	Control Animals	Colitis Control	A. L. E. G. S.	Treatment Control
Red Pulp Area	Normal	Increased	Normal	Increased
Red Pulp Cellularity	Normal	Increased	Decreased	Decreased
White Pulp Area	Normal	Increased	Normal	Decreased
White Pulp Cellularity	Normal	Decreased	Normal	Decreased
Peri-Arteriolar Lymphoid Sheet	Normal	Undefined	Normal	Undefined
Marginal Zone	Normal	Undefined	Increased	Undefined
Fibrosis	None	Numerous	None	Few

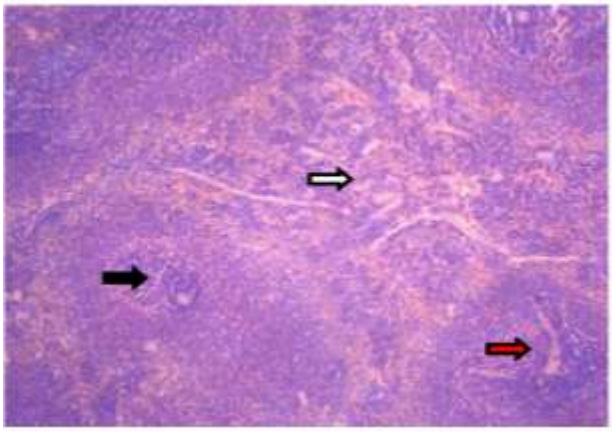

Figure 1: Photomicrograph of splenic tissue from control animal. Section shows normal lymphoid follicle (black arrow) and PALS (red arrow) in the white pulp. Red pulp (white arrow) appears normal (H&E. Mag. x100)

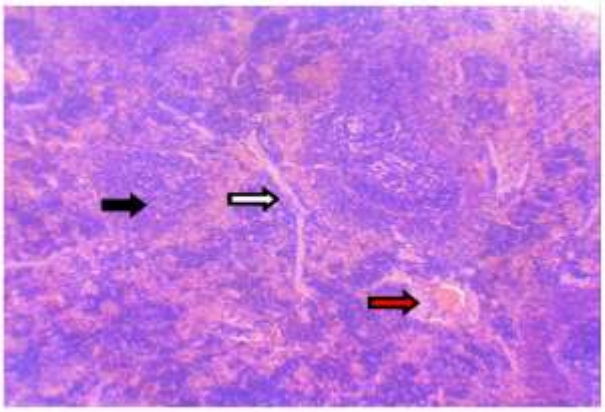

Figure 2: Photomicrograph of splenic tissue from colitis control animals. Section shows white pulp with numerous germinal centres and decreased cellularity (black arrow). There is a congestion of an artery in the red pulp (red arrow) with areas of fibrosis (white arrow). (H&E. Mag. x100).

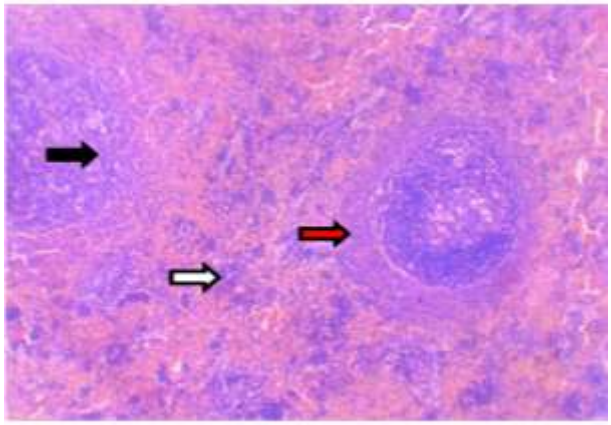

Figure 3: Photomicrograph of splenic tissue from animal receiving the extract. Section shows normal white pulp (black arrow) with an increase in cellularity of marginal zone (red arrow). Red pulp cellularity is slightly increased (white arrow). (H&E. Mag. x100).

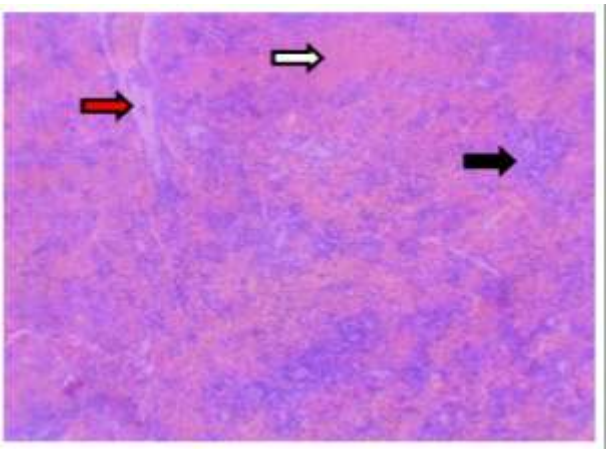

Figure 4: Photomicrograph of splenic tissue from treatment control animals. Section shows reduction in the cellularity of the white pulp (black arrow) and an expansive red pulp with reduced cellularity (white arrow). Areas of necrosis are also visible (red arrow) (H&E. Mag. x100).

DISCUSSION

Acetic acid induced colitis bears close resemblance to human IBD in terms of pathogenesis, histopathological features and inflammatory mediator profile.[19] and is therefore a reliable animal model that can be useful for evaluation of drugs for IBD.[20]

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.[7,14,21,22]

Due to variations in animal weight, the best marker for assessing weight loss is percentage decrease in body weight.[1,23] Abundant literature has asserted that acetic acid induced colitis is characterized by weight loss. Consequently, weight loss is a feature of IBD.[24] This experiment shows a dose dependent improvement in the weight of the animals during the study with no recorded weight loss in animals receiving 400mg/kg of the extract and those receiving prednisolone.

The spleen is the largest secondary lymphoid organ, 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.[25] as well as an important organ to evaluate for treatment-related lesions.[25,26] Acetic acid induced colitis was associated with splenic enlargement as seen in these findings. As spleen destroys unnecessary red blood cells and holds a reservoir of blood, systemic toxicity can be ascertained by its examination. The extract used in this study significantly decreased the splenic enlargement. Animals receiving 200mg/kg and 400mg/kg of the extract had a better mean splenic weight (0.56 and 0.52g respectively) than those receiving prednisolone (0.71g).

In any toxicologic study, spleen from the treated animals should be compared with age- and sex-matched control animals due to the variation in normal histological features that can be seen between sexes, strains and species of animals.[18] Furthermore, the spleen is considered the draining site for compounds that are administered,[10] and is therefore considered an important organ to evaluate for treatment-related lesions.[25] Due to the presence of B and T lymphocytes, the immunotoxic effects of xenobiotics or their metabolites on these cell populations may be reflected in the spleen. Therefore, it is one of the recommended organs to evaluate for enhanced histopathology of the immune system.[25]

Acetic acid induced colitis caused a reduction in cellularity of the splenic white pulp. It is seen as a direct treatment-related effect and can result as an indirect effect on body weight changes.[26] It also causes alterations in the red pulp morphology including fibrosis, apoptotic bodies and macrophages. This indicates a reparative process following inflammation or toxicity to the spleen.[26]

Administration of the extract caused an increase in cellularity of the white pulp in a dose dependent manner. This indicates an acute immune response,[25] and is a feature of immunomodulatory drugs.[18] The improvements in the lymphoid follicles (rich in B lymphocytes)[27] and the peri-arteriolar lymphoid sheath (rich in T lymphocyte)[27] also supports the immunomodulatory properties. Another supporting factor is the increase in the marginal zone of animals exposed to the extract indicating increased activity of marginal zone B cells which are notable for their varying roles in the immune system,[28] especially T-independent immune responses.[29]

The extract also caused a reduction in fibrosis of the red pulp and shows reduced areas of necrosis indicating healing function thus, supporting the works of other researchers.[14,30]

Treatment group showed an expensive red pulp with reduced cellularity. The white pulp was sparse with few follicles observed. A few areas of necrosis were observed and other features were undefined. This further confirms the immunotoxic and immunosuppressive effects of prednisolone on the spleen.[31,32]

CONCLUSION

The extract does not bear the side effects of immune suppression and lymphoid tissue toxicity that prednisolone have as evidenced by physical and microscopic evaluation of the spleen the safety of the extract as well as its immunomodulatory function.

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