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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Research Article

EVALUATION OF ANTI-ULCER POTENTIAL OF 80% ETHANOL EXTRACT OF *DESMODIUM CANESCENS* (L) DC. (FABAECAE) USING THE RAT MODEL OF ASPRIN-INDUCED ULCERS

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ABSTRACT

Desmodium canescens (L) DC (Fabaeceae) is used by the Chagga tribe of Kilimanjaro Region, Tanzania to treat peptic ulcers. This study aimed to evaluate the anti-ulcer potential of the ethanol extract of the aerial part of the plant. The extract was prepared by placing the plant materials in a glass percolator with 80% ethanol and allowed to stand at room temperature for 24 hours. The percolate was collected, filtered and concentrated by using a rotary evaporator at the temperature of 40°C. The extract so obtained was weighed and the yield was 14%. The toxicity of the extract was evaluated in mice and was found to be non toxic up to 5000mg/kg without any death. Phytochemical screening showed that, the 80% ethanol extract contains saponins, tannins, terpenoids, flavanoids and alkaloids. For evaluation of anti-ulcer activity, the crude ethanol extract of DC was orally administered to rats one hour before ulcer induction. The 80% ethanol extract of DC showed protection against aspirin induced gastric ulceration. The extract protected the rat stomach from ulceration by 76%, 94%, 96% at doses of (200, 350 and 600) mg/kg respectively ($P \leq 0.001$), as compared to solvent treated rats. Ranitidine, the histamine blocker, 30mg/kg protected the rat stomach by 100%. Pathological analysis of the rat stomach treated by DC extract showed that, there was increased protection of the epithelium lining of the rat stomach with increasing dose of the extract (protection in dose response manner). The results support the use of DC as a herbal medicine for peptic ulcers. The group of compounds found in the extract may have contributed to its anti-ulcer potential.

Keywords: *Desmodium canescens, toxicity evaluation, Anti-ulcer activity evaluation.*

1. INTRODUCTION

The incidence, prevalence and recurrence of peptic ulcers are still a global concern despite the existing conventional treatments. Peptic ulcers are caused by various factors, including two major ones that can cause disturbances to the mucosal resistance to injury thus resulting into peptic ulcers formation; the use of non-steroidal anti-inflammatory drugs (NSAIDs) and infection caused by *Helicobacter pylori* bacteria [1].

In the past two decades, health researchers have increased their efforts in traditional and alternative medicine in searching for new drugs for treating various ailments. Natural products have gained appreciation globally in traditional systems of fighting diseases. Plant sources are mostly used due to their perceived lower side effects, ease of accessibility and affordability. For instance, plants with traditional ethno-medicinal properties are used in peptic ulcer treatment and management by herbalists. There is, therefore, a need to evaluate such plants for their anti-ulcer potential [2].

D. canescens is a herb belonging to the family Fabaeceae. It has various uses including nutritional and medicinal purposes. It is claimed to treat peptic ulcers by the Chagga tribe of the Kilimanjaro region, Tanzania. Plants of the same genus are reported to have uses other than peptic ulcer treatment, including in western Kenya and Uganda where some species of this genus are used for the treatment of diarrhea, dyspepsia, asthma and abdominal pain [3]. According to this ethno botanical information, the genus Desmodium contains valuable medicinal plant species. *Desmodium gangeticum* has been studied and reported in India to have anti-ulcer activity [4]. Literature on most studied species of the genus Desmodium have revealed that it possesses natural histamine blockers such as soyasaponins which are important in peptic ulcer management [5]. The genus Desmodium contains about 350 species of which, only 30 species have been pharmacologically studied. Majority of the species are found in India and Africa. *Desmodium gangeticum* is more abundant in India than in Africa whereas *Desmodium canesces*is more abundant in Africa [6] and it is abundantly found in northern Tanzania, in Kilimanjaro region. However, there is limited or no ethno-botanical

information on *D. canescens*, and thus our current study pioneers to bridge this knowledge gap.

Peptic ulcer (PU) is a general term used to describe gastric (GU) and duodenal ulcers (DU). These names refer to the anatomical location where the ulcer is found. Such ulcers are due to injury of the epithelial layer in the gastric and duodenal mucosa. Both gastric and duodenal ulcers are due to corrosive action of pepsin and/or hydrochloric acid on the mucosa. Furthermore, peptic ulcers can develop in the esophagus besides the stomach and/or duodenum [7].

Medical therapy for PU aims to reduce acid secretion and the eradication of *H. pylori*. On one hand, a number of anti-ulcer drugs such as proton pump inhibitors and H2 receptor antagonists are used for the treatment of peptic ulcers, although they have been associated with incidences of relapses, side effects and drug interactions. On the other hand, herbs are gaining popularity in treating a number of disorders including peptic ulcers. Various herbs have been evaluated as therapeutic agents for the treatment of PU. Phytochemicals with principal anti-ulcer activity include flavonoids, tannins and terpenoids [8]. There is a need therefore to evaluate such plants for their anti-ulcer potential**.**

2. MATERIALS AND METHODS

2.1. Chemicals and drugs

Ranitidine injection, made by Induspharma, (pvt) Ltd, Karachi, Pakistan was purchased from a local pharmacy; Aspirin from Guangzhou Bayunshan pharmaceuticals Co. Ltd, Guangzhou China was purchased from the Keko Pharmaceutical Industry, Tanzania. Ethanol (absolute) was purchased from Fluka Chemie GmbH (Sigma-Aldrich®, Zwijndrecht, The Netherlands).

2.2. Plant material collection area

The aerial parts of DC were collected from agricultural fields at the primary school, Kiruweni Village, Moshi Rural District, Kilimanjaro Region in March 2016.This study area was selected due to existing literature that these plant materials have been used locally to treat the peptic ulcer disease. Several tools were used in the field, including perforated bags and a bush knife. A field press was also used to press the collected plant specimens before transportation to the lab at MUHAS; Voucher specimen no MEW001 is kept for reference.

2.3. Preparation and Extraction of the plant materials

The plant material was shade dried and ground. About 1.5kg of powdered plant was placed in a glass percolator with 80% ethanol and allowed to stand at room temperature for 24 hours. The percolate was collected. Extraction by percolation was repeated three times and the combined extract was filtered and concentrated by using a rotary evaporator at the temperature of 40°C. The extract so obtained was weighed and the yield of the plant material calculated.

2.4. Fractionation of the crude extract

Liquid-liquid fractionation was done by using a separating funnel using four different solvent of differing polarities; ethyl acetate, petroleum ether, dichloromethane and water. The factions were collected, concentrated in rotary evaporator. The water fraction was freeze dried.

2.5. Phytochemical Screening

2.5.1. Test for Tannins

Tanins were evaluated using 1% Ferric chloride solution as described by Trease and Avans, 2002. Occurrence of a blue-green precipitate indicated the presence of tannins.

2.5.2. Test for saponins

Saponins were tested by the frothing method as described by Sofowora, 1993 [9]. Frothing which persisted on warming was taken as an evidence for the presence of saponins.

2.5.3. Test for terpenoids

Terpenoids were tested by using conc. Sulphuric method as described by Sofowora, 1993 [9]. A change in colour from pink to violet showed the presence of terpenoids [9].

2.5.4. Test for flavonoids

Flavonoids were tested by sodium hydroxide solution method [10]. An intense yellow coloration which disappeared on the addition of dilute HCl suggest presence of Flavonoids [10].

2.5.5. Test for Alkaloid

Dragendoff test was used to screen DC crude extract for alkaloids [11]. Orange or yellow sports indicate presence of alkaloids.

2.5.6. Test for steroids

Steroids were tested by acetic acid and conc sulphuric

acid as described by Sofowora, 1993. Color development from violet to blue or bluish-green indicated the presence of a steroidal ring i.e. a glycone portion of cardiac glycoside [9].

2.6. Study design

This was an experimental study where lab animals (mice for acute toxicity and rats for anti-ulcer studies) were randomly allocated to groups. The animals were divided into 6 groups of 6 rats each. Each individual rat as well as their groups were marked. Control groups were given CMC 1% before oral administration of aspirin 500mg/kg. Treated groups were given DC extract one hour before oral administration of aspirin. All administered solutions were given at a dose volume of 10 ml/kg. Four hours after administration of the ulcerogen (aspirin), all animals were euthanized. The stomachs were then removed from the animals. Each stomach was opened along the greater curvature, pinned on flat surface. Data from individual stomachs was recorded in a separate raw data sheet and labeled accordingly.

2.7. Animals

Sprague Dawley rats of either sex weighing 70-110g and Swiss albino mice of either sex weighing 20 to 25g were obtained from the animal house of the Institute of Traditional Medicine (ITM) at the Muhimbili University of Health and Allied Sciences (MUHAS). Animals were kept in environmentally controlled rooms (25° ± 2°C, 12 h light and dark cycle). The animals were exposed to the same acclimatization condition before start of the experiment to allow them to acclimatize to the laboratory environment. Drinking water and food were provided throughout the experiment, except for fasting period where adequate drinking water was freely provided but no food supply was provided prior to treatment. Before administering the extracts, animals were starved in cages with wide wire mesh bottoms to prevent coprophagy.

2.8. Acute toxicity test

Acute toxicity study was done to determine safe dose of the DC. The study was done according to OECD guidelines 425. Limit test was used in this experiment because there was no literature which indicated that DC was likely to be toxic (having toxicity below regulatory limit doses.) The dose for each rat was calculated in reference to the body weight. The volume administered to a rat was 10mL/kg. The test extract was orally administered in one mouse in a single dose of 2000 mg/kg by gavage. The mouse was then observed for signs of toxicity, death or survival. Since this mouse survived, four additional mice were sequentially dosed with extract so that a total of 5 mice were used as no one among them died. Following this, one mouse was dosed 5000 mg/kg of the extract and observed for signs of toxicity, death or survival. Since this mouse survived, an additional mouse was dosed and observed again for 48hrs. Finally, an additional mouse was dosed with the extract and then observed for signs of toxicity, death or survival. All mice were observed in detail for any indications of toxicity effect within the first six hours after the treatment period, and daily for a period of 14 days.

2.9. Anti-ulcer activity

2.9.1. Aspirin induced ulcer

In this study, thirty-six young adult Sprague Dawley rats of either sex with weight between (70–110gm) were randomly picked from their cages then divided in six groups of six rats each. The first group was normal control group, which was given 1% CMC for normal comparison (neither ulcer nor treatment group). The second group was negative control, which was also given 1%CMC.The third group of rats was dosed with ranitidine30mg/kg to serve as positive control group. Fourth, fifth and sixth group were test groups orally treated with 200, 350 and 600 mg/kg of DC crude extract, respectively. Rats were fasted for 60 hours in order to produce distinct gastric ulcers capable of being quantified and treated one hour before induction of ulcers. Ulcers were induced by the administration of aspirin suspended in 1% CMC (at a dose of 500 mg/kg orally). After four hours, all rats were euthanized and their stomach opened along the greater curvature, washed carefully with normal saline and the number and severity of ulcers scored. This methodology was as described by Shah and Patel [12] with some modifications Ulcer scoring was done by the method described by Bhalke et al. [13]. The number and the severity of the ulcers scored were as follows:

0.5. Hemorrhage, 1. Streaks, 2. Spot ulcer, 3. Severe streaks, 4. Erosions, and 5. Perforation.

$$UI = UN + US + UP \times 10^{-1}$$

Whereby;

UI– ulcer index

UN – number of ulcers per animal

UP– Percentage of animals with ulcers

US - average severity score

The percentage protection was calculated using the formula proposed by Vijaya Kumar and Bongu [14] as follows:

% protection =(ulcer index) control-(ulcer index) test/ (ulcer index) control

2.9.2. Gross and Histological studies

After opening the abdomen, resecting out the stomachs and counting the ulcers, the stomachs were fixed in formalin and examined for macroscopic changes and/or development of any lesions as previously described [15, 16]. All of the tissue injuries were compared between both treated and control groups and these were histologically evaluated by a Histopathologist, at the Department of Pathology of the Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania and Muhimbili National Hospital (MNH).

After gross evaluation, stomachs from the rats were fixed for 24h in neutral well-buffered (40%) formalin, embedded in paraffin and sections (5µm) mounted on SuperFrost_ slides (Menzel GmbH &Co KG, Braunschweig, Germany) as previously described [15-17]. These were then deparaffinized, rehydrated and stained with haematoxylin and eosin (H&E) as previously described [17]. Histological evaluation and photomicrography was performed using an Olympus (CX31RBSF Model) light microscope equipped with a digital camera (Olympus Corporation, Tokyo, Japan).Ulcer induction activity was evaluated under the microscope on 7 low-power fields (x10 magnification) as well as on their high-power fields (x40 magnification) while taking pictures. Picture processing and printing was performed using Adobe Photoshop7.0 (Adobe Systems Incorporated, San Jose, CA, USA) and Microsoft- Power Point 2003 (Microsoft Corporation, Redmond, WA, USA) as previously described [15-17].

2.10. Statistical analysis

Statistical Package for the Social Sciences (SPSS) was used to run a one- way ANOVA for comparison of the test groups as well as Microsoft Excel spreadsheet application to assess the significant difference between groups. Confidence intervals (95%CI) were calculated.

2.11.Ethical Considerations

The protocol of the study was approved by the Ethics Committee (Institutional Review Board) of MUHAS and the use of mice in acute toxicity study and rats for anti-ulcer experiment followed guidelines for use of animals in experiments as adopted from internationally accepted principles for laboratory animal use and care (EEC Directive of 1986; 86/609/EEC)

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening Results

The results of phytochemical screening showed that, 80% ethanol extract of contains saponins, tannins, terpenoids, flavanoids and alkaloids as shown in Table 1.

Table 1: Results of preliminary phytochemical *Desmodium canescens* 80% ethanolic extract

Phytochemical Group	Results
Saponins	+
Tannins	+
Flavanoids	+
Akaloids	+
Terpenoids	+
Steroids	-

3.2. Acute oral toxicity

The limit test was done successfully. Mice were dosed (2000 and 5000) mg/kg. Eight mice were used in this study. All were carefully observed for the first six hours after administration of the test material and daily for 14 days without sign of toxicity or death. After 14 days the animals were thereafter sacrificed. There were no gross lesions observed in the major organs. LD_{50} of DC was estimated to be>5000mg/kg.

3.3. Results of anti-Ulcer activity

The oral administration of aspirin at dose of 500 mg/kg was efficiently able to induce quantifiable gastric ulcers in rats. The mean ulcer index of aspirin-induced ulcer was 13.80±0.76 for (negative control). Oral administration of three tested doses (200, 350 and 600mg/kg) was given to the rats one hour before aspirin treatment. The extract lowered the ulcer index significantly and increased the protection up to100% ulceration in a dose response manner. Similar protection was shown by ranitidine, the known H_2-histamine receptor antagonist, which was used as a positive control. Ranitidine showed good protection (100% reduction) against aspirin-induced ulcers as shown in Table 2.

Table 2: Effect of 80% ethanol extract of DC on ulcers index and percentage protection in rats

Dose(mg/kg)	Volume of gastric juice(ml)	PH of gastric juice	Ulcer Index	% Protection
Negative Control CMC 1% 10ml/kg	1.4±0.21	1.75±0.06	13.80±0.76	-
Positive control Ranitidine 30mg/kg	0.93±0.13	4.58±0.13	0.0±0.0	100
DC 200mg/kg	1.14±0.09	4.20±0.23	3.32±0.30	76
DC 350 mg/kg	2.03±0.36	4.55±0.27	0.77±0.25	94
DC 600mg/kg	1.78±0.22	4.44±0.455	0.52±0.10	96

Figure 1 below shows gross appearance of the rat stomachs treated by DC extract at doses of 200, 350 and 600 mg/kg respectively.

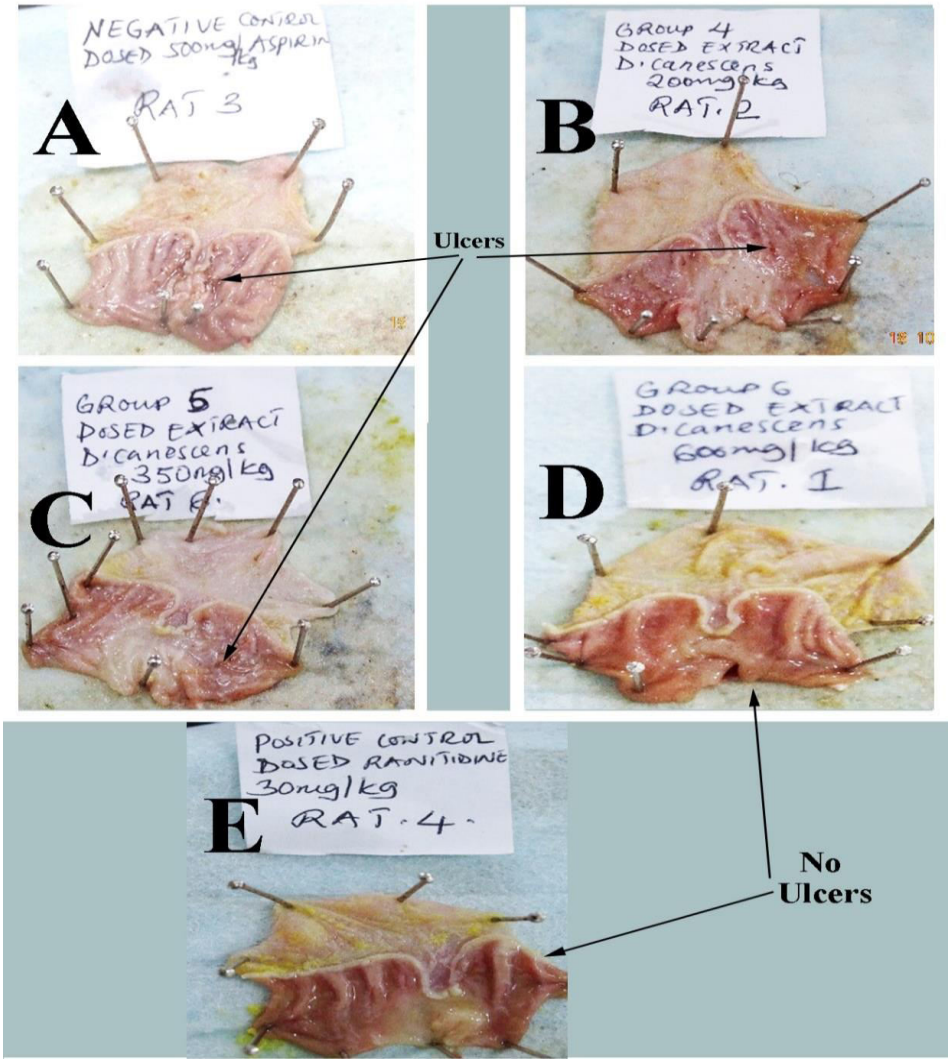

Fig. 1: Photograph showing protection of the rat stomach against aspirin induced ulceration following pretreatment by different doses of the DC crude extract

3.4. Histopathological analysis

Pathological analysis of the rat stomach treated by the DC extract showed that, there was increased protection of the epithelial lining of the rat stomach with increasing dose of the extract (protection in dose response manner). The pathological findings are as shown in Figure 2 and Table 3.

Table 3: Histological analysis of rat stomach after administration of the extract

	Normal control (no drug)	Negative Control (no drug)	Positive Control	Test drug	Test drug	Test drug
Rat No.	CMC 1% 10ml/kgbwt	CMC 1% 10ml/kgbwt	Ranitidine 30mg/kg	Dose =200 mg/kg	Dose =350 mg/kg	Dose = 600 mg/kg
1	Normal	Severe diffuse sloughing of gastric mucosa	Epithelial sloughing	Severe focal sloughing	Scanty sloughing	Normal
2	Normal	Moderate & Severe diffuse sloughing of gastric mucosa	Epithelial sloughing	More severe focal sloughing	Mild to moderate diffuse sloughing	Scanty focal Sloughing
3	Normal	Severe diffuse sloughing of gastric mucosa	Eosinophilia, parasites	Mild focal sloughing	Normal	Normal
4	Normal	Severe diffuse sloughing of gastric mucosa	Chatters, epithelial sloughing	Mild focal sloughing	Scanty focal sloughing	Scanty focal sloughing
5	Normal	Severe diffuse and focal sloughing of gastric mucosa	Focal epithelial sloughing	Mild focal sloughing	Normal	Normal
6	Normal with scattered eosinophilia	Severe diffuse sloughing of gastric mucosa	Hyperplastic mucosa with focal sloughing	Mild focal sloughing	Normal	Scanty focal Sloughing

4. DISCUSSION

The present study on DC is of particular importance due to its scanty literature. Our toxicity study results have shown that this plant is non-toxic in mice for a dose above 5000mg/kg body weight (LD_{50}>5000mg/kg). Toxicity studies done on other species of the same genus *Desmodium*, such as *Desmodium gangeticum* have revealed its LD_{50} to be greater than 2000mg/kg [18]. In the anti-ulcer activity test, aspirin induced gastric ulcer model was used. NSAIDs that induce gastric damage include indomethacin, aspirin, ibuprofen and diclofenac just to mention a few. They cause gastric ulcers especially when misused. This property has been employed in the development of Aspirin- induced gastric ulcer models in rats.

The model is important in investigating the potential usefulness of anti-secretory and cytoprotective agents due to underlying pathophysiology involved in gastric acid secretion and mucosal prostaglandin synthesis. It is the most commonly used ulcer model in anti-ulcer studies. The frequency of usage of NSAIDs especially aspirin may induce peptic ulcers. They are the second most common cause of peptic ulcers. It is the most frequently used ulcerogen in ulcer induction. Aspirin is known to induce ulcers by inhibiting prostaglandin synthetase in the cyclooxygenase pathway. Prostaglandins are found in stomach tissue where they play a vital protective role via stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal cell turnover and repair.

Thus, the suppression of prostaglandin synthesis by aspirin results in increased susceptibility to mucosal injury and therefore peptic ulcer. In the current study, ranitidine was used as positive control. It has shown protection of the rat stomach against aspirin induced gastric ulceration. Similarly, differences in appearance and protection of gastric mucosa were shown in stomachs of rats treated with (200, 350 and 600mg/kg) doses of ethanol extract of DC as compared to those of the negative control group.

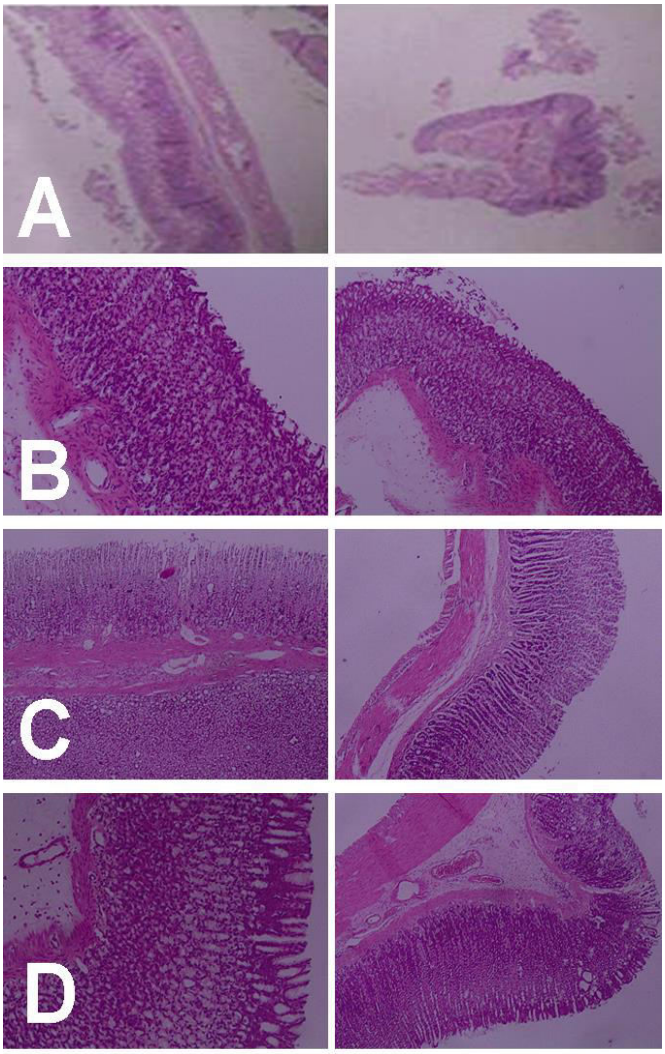

Fig.2: Photographs showing the histopathological analysis of the rat stomachs treated with the extract (A) Negative control, (B) Positive control (C) Pretreated by 200mg of DC extract (D) Pretreated by 600mg of the DC extract respectively.

Furthermore, the results of pathological analysis for the rat stomach treated by DC extract have shown protection of the gastric mucosa against ulceration. It has shown protection in a dose response manner. Phytochemical screening results from the DC 80% ethanol extract have shown that it contains saponins, tannins, terpenoids flavanoids and alkaloids. These compounds are known to have free radical scavenging, and cytoprotective properties [19]. Other studies on phytochemical and pharmacological activities on various *Desmodium* species revealed that they have secondary metabolites with anti-ulcer activity, including, triterpenoid saponins and soyasaponins which have been found in some studied species such as *Desmodum gangetcum* and *Desmodum adescendens*. They are reported to have anti-histamine and histamine blocker activity [20]. Probably these groups of compounds are contributing to the anti-ulcer activity of the DC extract.

5. CONCLUSION AND RECOMMENDATIONS

The DC crude extract at doses 200 mg/kg, 350 and 600 mg/kg was found to significantly inhibit ulcerogenicity in the aspirin-induced gastric ulcer model (76, 94 and 96 % respectively). This protective effect might have been due to the presence of terpenoid, tannins, saponins, flavanoids and alkaloids in the extract. These groups of compounds are known to contain antioxidant and anti-secretory properties. Due to the results obtained, it is recommended that, more long term studies for safety and identification of active compound(s) be done in order to promote the use of this herb for formulations for the management of peptic ulcers.

6. REFERENCES

1. Borrelli F, Izzo AA. *Phytotherapy Research,* 2000, 14; **8:**581-591.
2. Adinortey MB, Ansah C, Galyuon I, Nyarko A. *Ulcers,* 2013, **(796405)**:12.
3. Tabuti JR, Kukunda CB, Kaweesi D, Kasilo OM. *Journal of Ethnobiology and Ethnomedicine*, 2012, 8; **1:**35.
4. Dharmani P, Mishra PK, Maurya R, Chauhan VS, Palit G. *Indian Journal of Experimental Biology,* 2005, 43; **6:**517-521.
5. Martini L, Solimé R. *Our Dermatology Online*, 2014b 5; **1:** 29-32.
6. Ma X, Zheng C, Hu C, Rahman K, Qin L. *Journal of Ethnopharmacology*, 2011, 138; **2:**314-332.
7. Ramakrishnan K, Salinas RC. *Am Fam Physician,* 2007, Oct 1; 76; **7:**1005-1012.
8. Sharma D,Bhatt S. *International Journal of Pharmacy and Pharmaceutical Sciences,* 2014, 6; **10:**2-19.
9. Sofowora Abayomi, Medicinal Plants and Traditional Medicine in Africa. Spectrum Books 1993 (Book). ISBN 9782462195, 9789782462190.
10. Sharma V, Agarwal A, Chaudhary U, Singh M. *International Journal of Pharmacy and Pharmaceutical Science,* 2013, 5; **(1):**317-20.
11. Saraf A. *Journal of Chemistry,* 2010, **7; S1:**S405-S413.
12. Shah JS, Patel JR. *AYU,* 2012, 33; **2:**314-316.
13. Bhalke RD, Giri MA, Anarthe SJ, Pal SC. *International Journal of Pharmacy and Pharmaceutical Sciences,* 2010, 2; **4:**206-208.

14. Vijayakumar S. *African Journal of Pharmacy and Pharmacology*, 2013, 7; **35:**2522-2527.
15. Kalala W, Mwakigonja A, Maregesi S, Msengwa Z Mahunnah R. *Pyrex Journal of Medicinal Plant Research,* 2015, **1; 3:**010-018.
16. Maregesi SM, Mwakigonja AR, Peter Urio. *Sch. Acad. J. Pharm.,* 2016, 5; **10**:399-405.
17. Mwakigonja AR, Pak F, Pyakurel P, Mosha IJ, Urassa WK, Kaaya EE, Biberfeld P. *Oncol Rep.,* 2007, 17; **6:**1291-99.
18. Govindarajan R, Asare Anane H, Persaud S, Jones P, Houghton PJ. *Planta Medica,* 2007, 73; **5**:427-432.
19. Tsai J, Huang G, Chiu T, Huang S. *African Journal of Pharmacy and Pharmacology*, 2011, **5;** 4:468-476.
20. Rastogi S, Pandey MM, Rawat AKS. *Journal of ethnopharmacology*, 2011, 136; **(2)**:283-296.