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ANTI-ULCER AND WOUND HEALING ACTIVITIES OF *SIDA CORYMBOSA* IN RATS

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Abstract

Background: There are strong beliefs in the efficacy of traditional medical systems worldwide. Many herbs have been acclaimed to possess antiulcer effects and could be unexplored sources of new lead compounds. *Sida corymbosa* R. E. Fries *(*Malvaceae) is used in Northern Nigeria to treat ulcers and wounds. This work aimed to investigate the usefulness of *Sida corymbosa* in treatments of stomach ulcers and wounds in traditional medicine.
Materials and Methods: Effect of the aqueous extract was determined on gastric ulceration, rate of wound healing and inflammation using ethanol-induced and diclofenac-induced ulceration, wound excision model and albumin-induced inflammation respectively in rats.
Results: The study demonstrated the anti-ulcer activity of *Sida corymbosa* as the extract (250, 500 and 1000 mg/kg) showed a dose-dependent, significant ($P<0.05$) reduction of ulcer indices against gastric ulcers induced by both ethanol and diclofenac. Topical application of a formulation prepared with the extract of *Sida corymbosa* on surgically created incisions produced an increase in the rate of healing of the wounds. The extract of *Sida corymbosa* exhibited a significant ($P < 0.05$), dose-related decrease in inflammation induced by fresh egg albumin. This study showed that *Sida corymbosa* has constituents with the ability to reduce the severity of haemorrhagic gastric lesions, promote wound healing and reduce inflammation. These actions may be attributed to any one of the active constituents or as a result of synergistic effects of these phytoconstituents.
Conclusion: This study validates the use of the plant in traditional medicine for the treatment of stomach ulcers and wounds.

Keywords: *Sida corymbosa*, Anti-Ulcer, Wound Healing

Introduction

There are strong beliefs in the efficacy of traditional medical systems worldwide. The increasing interest in traditional medicines in both developed and developing societies has been attributed to the economic advantage provided by traditional medicines and the accessibility and assumed safety they offer when compared to conventional medicines (Osemene *et al*, 2011; Kunle *et al*., 2012). Several herbs have been acclaimed to possess antiulcer effects (Borrelli and Izzo, 2000; Bhattacharya *et al*., 2007; Alebiosu *et al*., 2012). These could serve as pool of unexplored sources of new lead compounds for the development of new drugs (Fabricant and Farnsworth, 2001; Vijayalakshmi and Ravindhran, 2012). There is therefore a need for scientific validation of these claims.

Sida corymbosa R.E. Fries Malvaceae commonly known as 'miyar tsanya' or 'karkashin kwado' in Northern Nigeria is a weed of cultivated fields, waste areas, road sides and open areas. The plant is an erect, basally woody perennial shrub with hairy stem of up to 2m high that reproduces from seed and occurs widely in Nigeria (Akobundu and Agyakwa, 1998). In Northern Nigeria, it is used to treat stomach ulcers and wounds and Ekpendu (2003) documented the use of the plant in the Benue area of Nigeria as an ulcer remedy. A survey of the literature did not yield result, pointing to the lack of scientific publication on gastro-protective action or wound healing effects. This present study was designed to establish the usefulness of *Sida corymbosa* in the treatment of ulcers and wounds in traditional medicine.

Materials and methods

Collection of plant material

The plant was collected by Mal I. Muazzam, then identified and authenticated by Mrs Grace Ugwabe (an ethno-botanist) both of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja where a herbarium specimen (NIPRD/H/6602) was deposited for future reference.

Preparation of the plant material

The leaves were air-dried in the shade and pulverised to obtain a coarse powder using a pestle and mortar. 200g of the powdered leaves were subjected to cold maceration extraction in water 1L with occasional shaking for 24 h. The filtrate was freeze dried to give a dry residue. It gave a yield of 9.47 % w/w.

Phytochemical screening

The extract was screened for the presence of tannins, saponins, alkaloids, flavonoids, carbohydrates, terpenes and sterols according to standard procedures (Odebiyi and Sofowora, 1978).

Formulation preparation

The formulation components used are as in Table 1. In the fume hood while using a hot water bath, the polar ingredients (Water and Extract) were heated in a 100 ml beaker for 5 minutes and set aside. The non-polar ingredients, lanolin, stearic acid and mineral oil were mixed at a temperature of 75 ^{o}C ± 5 ^{o}C in a separate beaker. The molten non-polar ingredients were slowly poured into the beaker containing the polar ingredient (Water). The mixture was stirred continuously until a smooth uniform paste was formed.

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Table 1: Formulation components of *Sida corymbosa* cream

	Cream base	C1	C2	C3
Stearic Acid (g)	5	5	5	5
Lanolin (g)	4	4	4	4
Mineral Oil (ml)	5	5	5	5
Water (ml)	25	25	25	25
Extract %	-	2.5	5	10

Components for the formulation of the aqueous leaf extract of *Sida corymbosa* cream.

Animals

Adult Wistar rats maintained at the Animal Facility Centre of NIPRD were used in this study. They were fed with mouse cubes (CAPS Plc. Nigeria Limited) and had access to water *ad libitum*. They were housed under standard condition of constant temperature; humidity and a 12 h light/dark cycle were maintained. All experiments were conducted according to the National Institute for Health guide and care for the use of laboratory animals (NIH publication No 80-23, revised 1985 as contained in NIPRD's Standard Operating Procedures for laboratory use of animals (NIPRD-SOP No O5:06).

Acute toxicity studies

Oral acute toxicity (LD_{50}) of the extract was evaluated in Wistar Rats using the Lorke's (1983) method. Three groups of 3 rats in each group were administered 10, 100, and 1000 mg/kg of extract respectively. These were observed for 24 h for signs of toxicity and death. Based on the results of the first stage, another set of animals (n=1) were treated with 1000, 1600, 2900 and 5000 mg/kg and observed for signs of toxicity and mortality for 24 h.

Studies on gastric ulceration

In this study, adult Wistar rats were fasted of food for 24 h prior to the experiment but allowed free access to water. The rats were randomised and divided into 5 groups of 6 rats each. Groups 1- 3 were treated with extract at 250 500 and 1000 mg/kg p.o, group 4 received vehicle, while group 5 were administered Omeprazole 20 mg/kg. After 60 min, 1 ml of 96% ethanol was administered to each rat intra-gastrically via an orogastric cannula. One hour after ethanol administration, the animals were sacrificed under ether anaesthesia; the stomachs were dissected out and opened along the greater curvature, then carefully rinsed under a running tap, fixed with 10% formalin and the lesions examined using a hand lens. The number and the severity of erosions were scored according to the ulcer scoring scale of Magistretti et al., (1988). 0 = no lesion; 1 = 1 – 3 small lesions (10 mm length); 2 = 1 – 3 large lesions (≥ 10 mm length); 3 = 1 – 3 thickened lesions; 4 = more than 3 small lesions; 5 = more than 3 large lesions; 6 = more than 3 thickened lesions. The ulcer index was expressed as the sum total for each stomach and the percentage inhibition was calculated using the following formula:

$\%\ \text{Inhibition} = (UI_{control} - UI_{treated}) / UI_{control} \times 100.$

Studies in diclofenac-induced ulceration

Rats were fasted of food for 24 h but allowed free access to water. The rats were randomly placed into five groups of 6 rats each. Diclofenac 80 mg/kg was administered orally 1 h after drug treatment. The animals were euthanised with ether 5 h after diclofenac administration. Their stomachs were dissected out, opened along the greater curvature, rinsed under a slow running tap, and then fixed in 10% formalin. These were examined with hand lens and gastric ulcers were rated using the method of Evbuonwa and Bolarinwa (1990): 0 - Normal, 0.5 -Punctuate or Pin-point; 1.0 - Two or more small haemorrhagic ulcers, less than 3 mm in diameter; 2.0 - Ulcers greater than 3 mm in diameter; 3.0 - Several ulcers. The ulcer index was expressed as the sum total for each stomach and percentage inhibition calculated.

Determination of rate of wound healing

Wistar rats (180 – 200g) of both sexes were divided into 5 groups of 6 animals each. The animals were anaesthetised with intraperitoneal injection of ketamine 100 mg/kg. The back hairs of the rats were depilated by shaving and the area cleaned with 70% alcohol. An area of 20 X 20 mm was marked on the shaved dorsal thoracic central region, and the entire thickness of the skin from the marked area excised to obtain a wound of about 400 mm^2 using toothed forceps, a surgical blade and pointed scissors. Groups 1- 4 were topically treated as follows: Group 1 was treated with the formulation base, while groups 2 - 4 with the formulations prepared with the test extract at concentrations of 2.5%, 5% and 10% respectively. 100 g of formulation of *Sida corymbosa* was applied on each rat at every time of application. The animals were housed individually and treated once daily for 7 days starting from the day of wound creation. The wound area was measured at 3-days interval and compared with the wound area on day zero and the wound area calculated. The percentage wound healing was calculated using the formula:

% Wound Healing = (Initial wound area - Unhealed wound Area) / Initial wound area X 100.

Effect on albumin induced inflammation

The anti-inflammatory activity of the extract was tested using fresh egg albumin induced paw oedema in rats (Winter et al., 1962; Anosike et al., 2012). Inflammation was induced by injecting 0.1 ml egg albumin into the sub-planter surface of the right hind paw 60 min after extract (250, 500 1000 mg/kg. p.o) administration. The changes in volume (cm^3) of the hind paw were measured with a LETICA Digital Plethysmometer (LE 7500) immediately before injection of the phlogistic agent and at 30 min interval after the injection of the egg albumin up to a period of 120 min. The control rats received an equivalent amount of normal saline, while Naprozen 5 mg/kg served as reference. Values were expressed as mean difference in paw volume ± SEM. Percentage inhibition was calculated using the following formula:

% Inhibition = 100 (1-Vt/Vc),

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where Vt = volume of treated group; Vc = volume of control group.

Statistical Analysis

Results were expressed as mean ± SEM. Significance was determined using ANOVA. Results were regarded as significant at $P < 0.05$.

Results

Preliminary phytochemical screening revealed the presence of alkaloids, saponins, tannins, terpenes, sterols, flavonoids and carbohydrates. No lethality, coma, convulsions or tremors were recorded at 24hr and after 7 days of observation. Therefore, LD_{50} of *Sida corymbosa* was estimated to be greater than 5000mg/kg using the oral route. However, the animals showed decreased locomotor activity.

Effect on Ethanol induced gastric ulceration

Sida corymbosa extract at doses of 250, 500 and 1000 mg/kg showed a dose-dependent, significant ($P<0.05$) reduction of ulcer indices against gastric ulcers induced by ethanol exhibiting a percentage inhibition of 19.90, 51.97 and 77.19% respectively (Table 2).

Effect on Diclofenac induced gastric ulceration

The extract of *Sida corymbosa* similarly decreased ulcer indices in diclofenac-induced ulcers, producing an inhibition of 23.64, 41.37 and 88.51 % (Table 2). The effect was dose-dependent and significant ($P<0.05$).

Table 2: Effect of the aqueous leaf extract *Sida corymbosa* (SC) on ethanol-induced and diclofenac-induced gastric ulceration

Treatment	Dose (mg/kg)	Ethanol-induced ulcer		Diclofenac-Induced ulcer	
		Ulcer Index	% Inhibition	Ulcer Index	% Inhibition
Distilled water	10ml/kg	5.83 ± 0.17	-	16.92 ± 1.42	-
SC	250	4.67 ± 0.61	19.90	12.92±0.74**	23.64
SC	500	2.50 ± 0.42*	51.97	9.92± 0.66**	41.37
SC	1000	1.33 ± 0.33*	77.19	3.50± 0.67**	79.31
Omeprazole	20	0.67± 1.67*	88.51	1.42± 0.24**	91.61

Values presented as mean ± SEM, (n=6); *P<0.05, **P<0.01, significant compared to control
Effect of *Sida corymbosa* leaf extract on ethanol-induced and diclofenac-induced gastric ulceration. Values are ulcer indices presented as mean ± SEM (n = 6); *P<0.05, **P<0.01, significant when compared to control.

Effect on excision wound

Topical application of a cream preparation of the aqueous extract of *Sida corymbosa* on surgically created wounds produced an increase in the rate of healing of the wounds. On day18, 100% wound healing was observed in rats that were treated with the 5% and 10% preparation of the extract when compared to the control group (Figure 1). All through the experiment, all wounds were devoid of any form of purulent discharge.

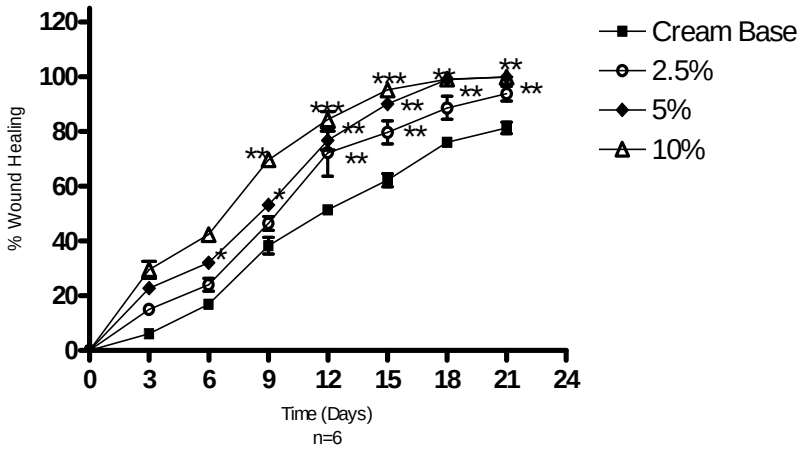

Figure 1: Effect of *Sida corymbosa* formulation on rate of wound healing of surgically created wounds

Effect of *Sida corymbosa* leaf extract cream on the rate of wound healing of surgically created wounds. Values are percentage of healing expressed as mean ± SEM (n=5); *P<0.05, **P<0.01, ***P<0.001when compared to control.

Effect on albumin induced inflammation

The extract of *Sida corymbosa* exhibited a decrease in inflammation induced by fresh egg albumin. The anti-inflammatory effect of *S.*

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corymbosa extract was evident with 1000 mg, showing greater inhibition of oedema in comparison with the other groups. This reduction in paw volume is dose related and significant (P<0.05) when compared to control (Table 3).

Table 3: Effect of *Sida corymbosa* (SC) on albumin induced inflammation

Treatment (mg/kg)	**Mean Difference in paw volume (% Inhibition)**			
	30 min	60 min	90 min	120 min
Distilled water	0.82 ± 0.02	0.72 ± 0.03	0.70 ± 0.03	0.69 ± 0.03
SC 250	0.71 ± 0.05 (13.41)	0.56 ± 0.03 (22.22)	0.53 ± 0.06 (24.29)	0.52 ± 0.05 (24.64)
SC 500	0.70 ± 0.05 (12.20)	0.60 ± 0.04 (16.67)	0.49 ± 0.05* (30.00)	0.42 ± 0.05* (39.13)
SC 1000	0.54 ± 0.05* (34.15)	0.48 ± 0.03* (33.33)	0.41± 0.04** (41.43)	0.30 ± 0.05** (56.52)
Naprozen 5	0.58 ± 0.02* (40.00)	0.50 ± 0.03* (30.56)	0.40 ± 0.04** (42.86)	0.26 ± 0.03*** (62.32)

Values presented as mean ± SEM, (n=6); *P<0.05, **P<0.01, P<0.001 significant compared to control.
Effect of *Sida corymbosa* leaf extract on fresh egg albumin induced paw oedema. Values are mean difference in paw volume (percentage inhibition) expressed as mean ± SEM (n=5); *P<0.05, **P<0.01, ***P<0.001when compared to control.

Discussion

The present study investigated the activity of the extract of *Sida corymbosa* on gastric ulcers induced by ethanol and the non-steroidal anti-inflammatory agent, diclofenac. The effects on albumin-induced inflammation and rate of healing of surgically induced wounds were also determined. Ethanol and NSAIDs are agents widely used to induce experimental gastric ulcers. The NSAIDs have been reported to cause interference in the cyclo-oxygenase pathway leading to the inhibition of prostaglandin production (Shakeerabanu et al., 2011). Prostaglandin E_2 and I_2 are the major prostaglandins involved with the maintenance of the integrity of gastric mucosa by inhibiting the production of gastric acids while promoting the secretion of mucous, bicarbonate and phospholipid secretion in gastric epithelial cells (Devi et al., 2007). Anti-oxidation studies have shown that oxygen derived free-radicals are implicated in the development of gastric lesions induced by ethanol and NSAIDs (Ray et al., 2002; Bharti et al., 2010). Free radicals have been associated with many human ailments including peptic ulcers; they are deleterious to biological tissues and may provoke the damage of these tissues (Bafna and Balaraman, 2011). This study demonstrates the anti-ulcer activity of *Sida corymbosa*. There was a marked reduction in the severity of gastric mucosal damage exhibited by a reduction of the number and size of haemorrhagic lesions in the stomach of treated rats. It is possible that the protective effect of *Sida corymbosa* may be related to an increased production of prostaglandins as Cho et al., (1987) and Nwidu and Nwafor (2009) have suggested that gastric mucous protection by non-prostanoid compounds may be triggered by the activation/congregation of endogenous gastroprotective mechanisms or compounds such as prostaglandins, surface active phospholipids, mucosal blood flow, growth factors and mucous-bicarbonate barrier. Previous studies have likewise proposed that substances that protect the gastric mucosa from the damage induced by ethanol may be enhancing the endogenous defence mechanisms (Mahmood et al., 2005).

Many phyto-chemicals have been attributed with anti-ulcerogenic properties (Sen *et al*., 2009). Phytochemical analysis showed the presence of alkaloids, saponins, tannins, terpenes, sterols, flavonoids and carbohydrates. The tannins, flavonoids, saponins, alkaloid and terpenes which are present in *Sida corymbosa* may be playing a contributory role in ameliorating the necrotoxic effect of the ulcerogens as these phyto-constituents have been associated with gastric mucosal protection in other plants (Nwafor et al., 2000; Mahmood et al., 2005; Yoshikawa et al., 2005; De Sousa Falcao et al., 2008; Souza et al., 2011). Besides augmenting endogenous defence mechanism, these plant constituents have the ability to scavenge free radicals, thereby protecting the gastric mucosa (Khalaf et al., 2008).

In this study *Sida corymbosa* formulation promoted wound healing by shrinking the wound area and reducing the epithelialisation period. Wound healing involves multiple intricate processes from vasoconstriction and platelets accumulation to produce a fibrin clot (haemostasis), followed by inflammation resulting from vasodilatation and phagocytosis. This is followed by the proliferative stage exhibited by angiogenesis, collagen deposition, wound contraction and epithelialisation to the remodelling stage consisting of the formation of new collagen and increase in tensile strength of the newly formed tissues (Wilgus, 2008; Badri and Renu, 2011). Wound recovery involves aggregation of skin tissues from the area surrounding the wound to repair the exposed area, an action which has been attributed to myofibroblast activity (Garg and Paliwal, 2011**).** The increase in the rate of wound healing caused by the extract of *Sida corymbosa* may therefore be as a result of increase in the concentration and movement of fibroblast surrounding the wound region or facilitation of the proliferation of epithelial cells to the uncovered area (Shanbhag *et al*., 2006). The formulation of the extract into a suitable preparation is to facilitate stability of the plant material and ensure even application unto the wound surface.

Lipid peroxidation has been proposed to be the mechanism by which oxygen free radical causes tissue damage (Sens *et al*., 2009). The presence of flavonoids in *Sida corymbosa* may partly be contributing to the healing process by inhibiting lipid peroxidation, thereby preventing tissue damage as oxidative stress has been associated with acute and chronic inflammatory conditions (Shuvaev and Muzykantov, 2011). Tannins and flavonoids by their astringent, antimicrobial and free radical scavenging properties promote wound healing (Soni and Singhai, 2012; Udobrel *et al*., 2012). These phytochemicals have been ascribed with the potential to regenerate and organise new tissues (Yusufogu, 2011). The extract of *Sida corymbosa* exhibited a decrease in inflammation induced by fresh egg albumin. Inflammation in tissues and organ systems is a process caused by the release of mediators such as histamines, prostaglandins, leukotrienes, bradykinin and thromboxanes. Inflammation process is the immune system response to infection and injury, although it has been implicated in the pathogenesis of several diseases and associated with conditions such as ulcers and wounds. Naturally occurring substances that promote wound healing with anti-inflammatory action may be of worth in wound care as their effects are mediated by promotion of natural wound healing mechanisms (Khan *et al*., 2004). Although the mechanism of anti-inflammatory effects cannot be ascertained from these experiments, it can be speculated to be due to the presence of phytochemicals with anti-inflammatory and free radical scavenging capacity (Tadic *et al*., 2008). LD_{50} of *Sida corymbosa* was estimated to be greater than 5000mg/kg using the oral route. This indicates that the extract has low toxicity and is practically safe when administered orally (Nguide *et a*l, 2013).

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This study has shown that *Sida corymbosa* has constituents with the ability to reduce the severity of haemorrhagic gastric lesions, reduce inflammation and increase the rate of wound healing in rodents. These actions may be attributed to any one of the active constituents or as a result of synergistic effects. Although the study does not explain the exact mechanism(s) of action involved, the use of the plant in traditional medicine for the treatment of stomach ulcers and in wound care is validated. Studies are ongoing to determine the mechanism(s) of action involved.

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