

Wound Healing and Anti-Ulcerogenic Activity of *Gardenia angustifolia* Extract in Rats

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Abstract

Gardenia angustifolia is widely utilized in many parts of Nigeria to manage a wide range of ailments. As part of effort to elucidate its pharmacological activities and hence medicinal potential, wound healing and anti-ulcerogenic properties of the extract was evaluated using experimentally created wound and ulcers in albino rats. Wound healing properties was evaluated using excision wound model, while anti-ulcer activity was studied using ethanol induced ulcer model. Five groups of rats were experimentally wounded at the back area. An area of uniform wound of 7×7 mm using millimeter ruler was excised. The animal groups were topically treated with *Gardenia angustifolia* gel, wound dressed with leaf, fruit and root gel significantly healed earlier than those treated with paraffin base and povidone iodine (standard). In anti-ulcer studies, rats were orally administered with different doses of the root extract (100, 250 and 500 mg/kg body weight) and positive control group (Omeprazole, 8 mg/kg body weight) for five days. After induction of ulcer using 5 ml/kg body weight of ethanol, the stomach of the rats was opened, gastric volume and ulcer area were measured. The results indicated that *Gardenia angustifolia* root extract could prevent ulceration in rats in a dose dependent manner. The acute toxicity study revealed that the plant could be toxic at higher doses. Blood glucose reduction was dose and time dependent. From this study it is evident that *G. angustifolia* possess anti-ulcer properties and also wound dressed with the root, leaf and fruit gels significantly enhanced the acceleration of wound healing in rats.

Keywords: *Gardenia angustifolia*; Anti-ulcer; Wound healing; Albino rats; Omeprazole; Toxicity

Introduction

Wound is a breach in the normal tissue continuum, resulting in a variety of cellular and molecular sequelae. The basic principles of optimal wound healing which include minimizing tissue damage, debriding nonviable tissue, maximizing tissue perfusion and oxygenation, proper nutrition and moist wound healing environment have been recognized for many years [1].

A number of drugs ranging from simple non-expensive analgesics to complex and expensive chemotherapeutic agents administered in the management of wound affect healing either positively or negatively [2]. Aspirin, indomethacin, cytotoxic agents and immunosuppressant have been proved experimentally to affect healing negatively [3]. Wound is defined as the disruption of the cellular and anatomic discontinuity of a tissue and may be produced by chemical, physical, thermal, microbial or immunological insult to the tissue. Wound cause discomfort and are more prone to infection and other troublesome complications [4].

Peptic ulcer is a conglomerate of heterogeneous disorders, which manifests itself as a break in the lining of the gastrointestinal mucosa bathed by acid and or pepsin [5]. Mucosal injury may occur when noxious factors “overwhelm” an intact mucosal defense or when the mucosal defense is somehow in paired [6]. Although a number of anti-ulcer drugs such as H₂ receptors antagonists, proton pump inhibitors and cytoprotectants are available for ulceration all these drugs have side effects and limitations. Herbal medicine deals with plants and plant extracts in treating diseases. These medicines are considered safer because of the natural ingredients with no side effects [7]. These natural agents induce healing and regeneration of the tissue by multiple mechanisms, however, there is need for scientific validation, standardization and safety evaluation of plants of traditional medicine before they could be recommended for wound and ulcer treatment [8].

Gardenia angustifolia (*G. angustifolia*) is locally called *Ikaga* by the Igala people of Kogi State, Nigeria. *G. angustifolia* (*Rubiaceae*) is

found in the tropical and subtropical regions of Africa and Southern Asia. Different parts of *G. angustifolia* are used in the management of many diseases traditionally including abdominal irritation, abortion abscesses, chicken pox, mental disorder, erectile dysfunction, cough, diabetes, etc. In spite of being one of the well-known medicinal plants used in Nigerian traditional medicine, studies pertaining to the pharmacological properties of *G. angustifolia* are very rare. There is no scientific evidence available for wound healing and anti-ulcer activity of *G. angustifolia*; therefore, the present research was undertaken.

Materials and Methods

Plant material

The plant sample was collected from Abocho in Dekina Local Government Area of Kogi State, Nigeria during rainy season when the plant thrives well and produces fruit. Dirt was removed from the plant sample by rinsing properly in clean water. The sample was air-dried for three weeks and pulverized into powder form using mechanical hand blender and sieved.

Preparation of extract

Portions of the samples-600 g of the powdered root, 300 g of the leaf and 60 g of the powdered fruit were weighed out and soaked in containers containing 1200 ml, 800 ml and 200 ml of methanol

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respectively for 72 hours. The mixtures were then filtered under vacuum pressure and the filtrates were concentrated by evaporation using water bath at 60°C.

Animals

The animals selected for this study were albino rats of both sexes with average weight range between 130–220 g. They were obtained from Mr. Friday Titus Emmanuel who breeds them and housed in the departmental animal house during the experimental period in a standard environmental conditions. They were allowed free access to standard laboratory diet and drinking water without any form of restriction. This study was carried out in conformity with national and international laws and guidelines for care and use of laboratory animals in biomedical research; especially as promulgated and approved by United States Institute of Health (1985).

Ointment preparation for topical application

Methanol-free extract of *G. angustifolia* leaf, root and fruit gels were used for the preparation of the ointment for topical application [9]. A 50% (w/w) of extract ointments of *G. angustifolia* (leaf, root and fruit) were formulated using soft white paraffin base [10].

Excision wound model

Animals were anaesthetized with 0.3 ml of lindocane adrexyll (local anesthetic) to prevent any movement of animals for at least two hours after administration and animals were left without being restrained [11].

The back of the animals were shaved and sterilized with 70% ethanol before 7×7 mm excision wound was created by a surgical blade from a predetermined shaved area on the back of each animal [12]. The experimental groups were topically applied with the extracts twice daily for consecutive 16 days. The group treated with povidone iodine drug served as a reference-standard. A progressive decrease in the wound area was periodically monitored. The wound contractions or closures were measured by a tracing paper on the wounded margin and calculated as percentage reduction in wounded area. The actual value was converted into percentage value taking the size of the wound at the time of wounding as 100%. The animals were divided into five groups of four animals per group.

Group 1: Control (wound treated with paraffin base).

Group 2: Standard (wound treated with povidone iodine).

Group 3: Wound treated with *G. angustifolia* root extract ointment.

Group 4: Wound treated with *G. angustifolia* leaf extract ointment.

Group 5: Wound treated with *G. angustifolia* fruit extract ointment.

Measurement of wound closure was taken the 4, 6, 8, 10 and 16th day post wound creation.

Acute toxicity

Seven groups (n=7) of albino rats of both sex were used in the acute toxicity of the root extract of *G. angustifolia*. Animals from all groups were fasted overnight and a safe dose of extract was determined through the acute oral toxicity test in rats at different doses up to 6000 mg/kg⁻¹ was prepared by dissolving the extract in phosphate buffer saline (PBS). The extract was then administered (P.O.) with single dose (100, 200, 500, 1000, 2000, 5000 and 6000 mg/kg⁻¹) of the extract. The animals were observed for behavioral changes, any sign of toxicity and

mortality up to 48 hrs and their blood glucose level were measured after 12 and 24 hrs. Blood glucose measurement: The blood glucose level was determined according to the method described by [13].

Anti-ulcer activity study

Five groups (n=4) of albino rats were used to evaluate the anti-ulcerogenic activity of *G. angustifolia* methanol extract. Phosphate buffer saline (PBS), *G. angustifolia* methanol extract, Omeprazole and ethanol were administered to the group of animals per orally (P.O.). Group 1 received PBS (10 ml/kg⁻¹) for 4 days and on the 5th day received absolute ethanol (5 ml/kg⁻¹) and served as ulcer control. Group 2 was administered Omeprazole (8 ml/kg⁻¹) for 5 days and served as positive control. Group 3, 4 and 5 were respectively administered with 100, 250 and 500 ml/kg⁻¹ of *G. angustifolia* methanol extract for 5 days. All groups were fasted for 24 hrs and again administered with the extract or drug at the respective dose. After 30 minutes of this treatment, animals of group 2-5 were administered with 5 ml/kg⁻¹ ethanol to induce ulcer. After 15 minutes of ethanol administration, all the animals were sacrificed using anaesthetic ether. Gastric volume was measured by pylorus ligation approach [14].

Each animal's stomach was opened along the greater curvature and examined macroscopically for gastric erosions under a dissecting microscope (20x). The length and width (mm) of ulcer on the gastric mucosa were measured by plane glass square (10x10mm). The ulcer area (UA) was calculated. The percentage of protection (P%) availed the animals through various treatments which are calculated using the formula:

$$P\% = \frac{(UA \text{ Ulcer Control} - UA \text{ Treatment})}{UA \text{ Ulcer Control}} \times \frac{100}{1}$$

Chemicals and reference drugs

All chemicals and reagents used in this investigation were of analytical grade and were obtained from BDH, Poole, England. Omeprazole and povidone iodine (reference drugs) were obtained from Kuzak Pharmacy in Anyigba, Kogi State, Nigeria. Omeprazole is an anti-ulcer drug which blocks the enzymes in the wall of the stomach from producing acid, the main culprit in peptic ulcer. By blocking the enzymes, the production of stomach acid is decreased, thus allowing the ulcer to heal [15].

Statistical analysis

All data were expressed as mean ± SEM. Statistical comparisons were performed using ANOVA. The level of significance was set at P<0.01.

Results

Wound healing

The topical application of *G. angustifolia* ointments increased the percentage of wound closure and this indicates rapid epithelization and collagenation. The administration of the extracts (root, leaf and fruit) accelerated the progression of wound healing. The root extract appears more potent than the leaf and fruit ointments (Table 1).

Acute toxicity evaluation

The result is as presented in table 2. Single dose (100, 200, 500, 1000, 2000, 5000 and 6000 mg/kg⁻¹) of *G. angustifolia* root methanol extract administered to albino rats elicited some behavioural characteristics in them. There were some physical signs of toxicity when doses up 500

Group/Treatment	Percentage Wound Contraction				
	4	6	8	10	16
1. Paraffin base	14.11 ± 0.35 ^a	21.26 ± 0.05 ^a	39.00 ± 6.97 ^a	49.50 ± 7.67 ^a	60.49 ± 6.77 ^a
2. Povidone iodine	17.69 ± 7.27 ^a	28.22 ± 0.42 ^a	42.68 ± 11.37 ^a	53.49 ± 7.09 ^a	71.24 ± 0.36 ^a
3. Root extract	21.24 ± 7.50 ^{ab}	30.73 ± 7.76 ^{ab}	48.53 ± 7.48 ^{ab}	64.38 ± 8.19 ^{ab}	87.36 ± 3.46 ^{ab}
4. Leaf extract	17.50 ± 7.85 ^a	28.39 ± 0.37 ^a	46.07 ± 6.91 ^{ab}	60.46 ± 7.12 ^{ab}	81.81 ± 7.12 ^{ab}
5. Fruit extract	21.07 ± 6.65 ^{ab}	24.75 ± 6.98 ^a	45.93 ± 7.11 ^a	49.49 ± 8.34 ^a	67.25 ± 6.74 ^{ab}

Values are expressed as mean ± S.E. The mean values with the same superscript (ab) in the same column are significantly different (P<0.01). Values in the same column with (a) superscript are not significant (P<0.01) when compared with the reference standard.

Table 1: Effect of *G. angustifolia* extracts on wound closure.

Dosage (mg/kg) Time (hr)	100		200		500		1000		2000		5000		6000					
	2	4	6	8	12	24	2	4	6	8	12	24	2	4	6	8	12	24
Gross activity																		
Respiration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Writhing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Convulsion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hind limb paralysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sedation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin irritation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eye irritation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CNS depression	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+: Indicates that changes were observed; -: Indicates that there was no change; D: Indicates death.

Table 2: Changes in the animal's behavior after administration of *G. angustifolia* methanol root extracts.

mg/kg⁻¹ and above were administered. Death was recorded at 6000 mg/kg⁻¹ dose (Table 2). Fast respiration, mild tremor, sleepingness, and depression were observed. These observations showed that the oral LD₅₀ of *G. angustifolia* is less than 6000 mg/kg⁻¹ in albino rats. The animals were able to overcome the effects after 7 days of post experiment.

Blood glucose

The plant extract elicits reduction in blood glucose and this is dose and time dependent as seen in table 3. This plant may be useful in the diabetic condition.

Anti-ulcerogenic activity study

As presented in table 4, *G. angustifolia* extract exhibited a dose-dependent protection from ethanol-induced ulceration. In comparison with the ulcer control group, *G. angustifolia* provided 48, 59 and 71% protection respectively at 100, 250 and 500 mg/kg⁻¹ dose. The reference standard, Omeprazole offered the highest protection at 8 mg/kg⁻¹ dose with 76.15% protection. The effect of 500 mg/kg⁻¹ dose of extract was quite comparable (71.37%) with the reference standard.

Discussion

The methanol extract of *G. angustifolia* was evaluated for wound healing activity using excision wound model in albino rats. The wound area measurement post wounding days (Table 1) showed that wound size of the test groups were reduced early as compared to control group. In addition to the reduction in wound size, the test group also showed a faster rate of healing. The order of increasing wound contraction of the various treatments is as follows:

Paraffin base → Fruit extract ointment → Povidone iodine
 → Leaf extract ointment → Root extracts ointment

The root extract showed the highest healing activity. Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissues as closely as possible to its normal state.

Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. In maturation phase, the final phase of wound healing the wound undergoes contraction resulting in a smaller amount of apparent scar tissue.

Granulation tissue formed in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, edema, and new small blood vessel [16]. In this study, it may be inferred that *G. angustifolia* ointments have the potential to satisfy all requirements of an ideal dressing material in that it provides an environment at the surface of the wound in which healing took place at the maximum rate consistent with the formation of granulation tissue with an acceptable cosmetic appearance and also provides a rationale for the use of *G. angustifolia* preparations in traditional system of medicine to promote wound healing.

Furthermore, it can be concluded that *G. angustifolia* extract has a beneficial effect as antiseptic and as an injury healing promoter. This effect may be explained by several mechanisms such as coating the wound, forming complexes with proteins of microorganism cell wall, chelating free radicals and reactive oxygen species, stimulating the contraction of the wound and increasing the formation of new capillaries and fibroblasts. Moreover, the extract did not produce any adverse effect on the wound surface and because of this it is possible to recommend its use in the treatment of skin wounds or ulcers.

Methanol extract of the root of *G. angustifolia* up to 5000 mg/kg⁻¹ did not cause any mortality in rats. All the doses below 5000 mg/kg⁻¹ did not produce any gross apparent effect on general motor activity; there was no convulsion, salivation, diarrhea and paralysis. Though there were mild signs of CNS depression at higher doses as well as sedation, respiration changes and slight tremor but all these signs faded as the time of exposure increased thus at 48 hrs after administration, the animals were near normal and well again (Table 2). Any substance that is not toxic at 5000 mg/kg⁻¹ is considered relatively safe [17].

Dosage (mg/kg ⁻¹)	Blood Glucose (mg/dl) after 12 Hrs	Blood Glucose (mg/dl) after 24 Hrs
100	66	52
250	65	50
500	61	54
1000	60	46
2000	58	49
5000	54	48

Table 3: Effect of *G. angustifolia* on blood glucose level in rats.

Treatment	Dose (mg/kg ⁻¹)	Gastric Volume (ML)	Ulcer Area (mm ²)	Protection (%)
Ulcer control	0	2.98 ± 0.22 ^a	753.33 ± 4.32 ^a	0.00
Drug control (Omeprazole)	8	1.46 ± 0.12 ^b	171.33 ± 8.62 ^c	76.15
Treatment 1 (<i>G. angustifolia</i>)	100	1.63 ± 0.03 ^c	379.33 ± 24.68 ^b	47.59
Treatment 2 (<i>G. angustifolia</i>)	250	1.67 ± 0.10 ^b	297.66 ± 23.16 ^d	58.65
Treatment 3 (<i>G. angustifolia</i>)	500	1.55 ± 0.62 ^c	191.67 ± 14.84 ^e	71.37

Values are expressed as mean ± S.E. Values in a column followed by different letters are significantly different (P<0.01). Values in a column with an asterisk (*) are significantly different from ulcer control (P<0.01).

Table 4: Anti-ulcerogenic effect of *G. angustifolia* root methanol extract.

Ulcer has long been recognized as one of the most important gastrointestinal problem. Peptic ulcers are common disorder of the entire gastro-intestinal tract that occurs mainly in the stomach and the proximal duodenum [18]. With the ever growing interest in natural medicine, many plants have been screened and reported to be useful in treating and managing ulcer. *G. angustifolia* has been claimed to have several pharmacological properties. In spite of its use in the traditional medicine against various ailments, this plant has so far not been screened for anti-ulcer activity. This report on its anti-ulcer activity appears to be the first in literature. The results of the present study have shown that *G. angustifolia* root extract possess gastro-protective activity, as evidenced by its significant inhibition in the formation of ulcers induced by ethanol (Table 4).

Since ulcer is a multi-factorial disease, its treatment faces great difficulties due to the limited effectiveness and severe side effects of the currently available drugs [18]. Owing to the side effects of the available drugs and cost, many plant-derived natural products have been evaluated as therapeutics for the treatment of a variety of diseases, including the peptic ulcer [19].

As presented in table 4, the methanol extract of the root of *G. angustifolia* (100, 250 and 500 mg/kg⁻¹) and Omeprazole (8 mg/kg⁻¹) significantly inhibited ulcer formation in this model by 47.59, 58.65, 71.37 and 76.15% respectively. The reduction of the lesions seen with the methanol extract of *G. angustifolia* suggests that part of the protective mechanisms could involve mucosal defensive factors. Gastric mucosal damage caused by ethanol and related non-steroidal anti-inflammatory drugs result from the inhibition of prostaglandins synthesis via the arachidonic pathway [20]. Prostaglandins serve protective functions in the stomach by maintaining gastric microcirculation [20] and causing gastric secretion of bicarbonate and mucus [21]. Thus, the effect of the extract in this model suggests that it may possess cytoprotective action, probably by enhancing prostaglandin synthesis. The extract significantly (P<0.01) protected gastric mucosa against ethanol challenge.

Ethanol-induced gastric mucosal lesions, predominant in the glandular part of the stomach are caused by the direct toxic action of

ethanol, reduction of the secretion of bicarbonate and depletion of gastric wall mucus [22].

Conclusion

In conclusion, our results suggest that *G. angustifolia* methanol extract ointment possess wound healing activity and gastro-protective effect against acute ethanol-induced ulcer models in rats. In this regard, we suggest that natural gastro-protective agent in *G. angustifolia* may be effective as plant gastro-protector and thus may have some obvious therapeutic implications. This plant could be toxic at higher doses. Further work is needed in the area of biosafety, phytoconstituents and mechanisms of action of *G. angustifolia* in wound healing action.

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