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Original Research

Toxicity status and antiulcerative potential of *Sansevieria trifasciata* leaf extract in Wistar rats

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ABSTRACT

Aims: The lethal dose 50% (LD_{50}) and antiulcerative potentials of *Sansevieria trifasciata* (ST) leaf extract were investigated. **Materials and Methods:** LD_{50} was determined through two routes of administration (intraperitoneal [i.p] and oral [p.o]) using the method of Lorke. The antiulcerative activity was evaluated in indomethacin-induced ulcer model (40 mg/kg body weight [BW], i.p, single dose) against a reference drug, cimetidine (100 mg/kg BW, p.o). ST was assessed at two different doses (200 and 400 mg/kg BW, p.o). Treatments were done twice daily at 8 h interval for 7 days before indomethacin administration. **Results:** The i.p LD_{50} was determined as 774.60 mg/kg BW and oral administration of the extract at 18,000 mg/kg BW dosage did not cause any negative behavioral changes in the animals, and no mortality was recorded after 24 h of the experiment. ST-pre-treated animals showed some improvement against indomethacin-induced ulceration. The extract curtailed indomethacin-induced reduction in gastric volume (36.1%), free acidity (55.3%), total acidity (35.6%) while minimizing the increase in pH by 13.3%. Moreover, the extract showed 17.92% and 14.96% ulcer protective ability at 200 and 400 mg/kg BW, respectively. The phytochemical analysis of ST extract revealed the presence of phytoconstituents such as glycosides, saponins, flavonoids, terpenoids, alkaloids, tannins, anthraquinone, and glycosides. **Conclusions:** ST apparently has a promising antiulcerative potential, and is safe for use in folk medicine. This valuable medicinal property is probably due to the array of important phytochemicals contained in the plant as observed in this study. However, a further study involving bioassay-guided identification of the main antiulcerative compound in ST is required to establish the use of the plant as a viable antiulcerative agent.

KEY WORDS: Gastric ulcer, indomethacin, *Sansevieria trifasciata*, toxicity status

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INTRODUCTION

Gastric ulcer remains a health burden on almost 10% of the world populace, and the need for antiulcerative agents from natural sources, such as plants is becoming popular and acceptable. This is probably because formulated drugs are relatively expensive and often associated with adverse side effects. Physiological imbalance between aggressive factors (pepsin and hydrochloric acid) and protective factors (bicarbonate and mucus) in the stomach is adjudged the primary cause of gastric ulcer [1,2]. Bad dietary habits, stress, *Helicobacter pylori* infection, and excessive use of nonsteroidal anti-inflammatory drugs (NSAIDs) are other factors which may result in gastric ulcer [3,4]. Patients with gastric ulcer have over the years relied on orthodox drugs such as cimetidine, ranitidine, antacids, and omeprazole for the management of the disease. However, in recent times, for different reasons, including those aforementioned there is an increased public interest in plant therapies [5].

Sansevieria trifasciata (ST) is one of the 70 species of *Sansevieria* genus. It is a species of flowering plant in the family Asparagaceae, native to tropical West Africa. The plant is often referred to as viper's bowstring hemp, snake plant, mother-in-law's tongue, or Saint George's sword (in Brazil). It has significant therapeutic utilization in folklore medicine [6]. In Africa, the plant is used as a protective charm against evil or bewitchment [7-9]. The use of ST in folk medicine for the treatment of different ailments such as ear-ache, ulcer, jaundice, pharyngitis, skin itches, urinary diseases, analgesic and antipyretic is well known [10]. This study, therefore, sought to evaluate the antiulcer potential of the ethanol leaf extract of the plant.

MATERIALS AND METHODS

Collection of ST Leaves

Fresh ST leaves were harvested in the month of December 2015 from Ibadan south west Local Government Area of

Ibadan, Nigeria. The harvested leaves were authenticated at the Department of Botany, University of Ibadan. Where a specimen was deposited and assigned a voucher number, UIH 22435.

Preparation of ST Leaf Extracts

The plant material was freed of extraneous materials; air dried at room temperature and milled to a fine powder, using a Warring blender. 300 g of the powdered sample was macerated in 2.5 L of the extracting solvents (ethanol) at room temperature (27 ± 2°C). The mixture was allowed to stand for 72 h and stirred intermittently with a glass rod to facilitate extraction. Sieving of the mixture was achieved with a muslin cloth (maximum pore size 2 mm). The resulting filtrate on sieving was further filtered through Whatman filter paper (No. 42) and subsequently reduced in volume with a rotary evaporator at 40°C. Final elimination of solvent and drying was done using a regulated water bath at 40°C.

Collection and Management of Animals

Male rats of the Wistar strain (102-151 g) were used for the study. The rats were purchased from the Animal Breeding Unit, Department of Anatomy, University of Ibadan. All procedures for maintenance and sacrifice (care and use) of animals were carried out according to the criteria outlined by the National Academy of Science published by the National Institute of Health [11]. The animals were handled humanely, kept in plastic suspended cages, placed in a well-ventilated and hygienic rat house under suitable conditions of room temperature (27 ± 2°C) and humidity. They were provided rat pellets with water *ad libitum* and subjected to a natural photoperiod of 12 h light and 12 h dark cycle. The animals were allowed 2 weeks of acclimatization before the commencement of all animal model experiments in this study.

Lethal Dose Determination of Ethanol Leaf Extract of ST

Lethality studies to determine the lethal dose 50% (LD_{50}) of the extract were performed according to the combined procedures described by Lorke [12] and OECD guidelines-425 [13]. It was assessed through two routes of administration; intraperitoneal (i.p), and oral (p.o). For i.p determinations, 40 male rats were randomly assigned to 10 groups, with each group having 4 animals. They were, respectively, treated with 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, and 2000 mg of the extract per kg body weight (BW) of the animals. The animals were then returned to their respective cages, allowed free access to pellets and drinking water 3 h later. They were thereafter monitored for clinical signs, symptoms, behavioral change, feeding pattern and mortality within 24 h of the experiment. Animals were observed individually once during the first 30 min after dosing, periodically during the first 24 h (with more attention during the first 4 h), and daily for a period of 14 days.

For the oral LD_{50} determination, three different sets of animals were used. The first set of animals was randomized into five groups, each containing four rats. They were treated with 1000, 2000, 3000, 4000, and 5000 mg/kg BW with no mortality recorded after 24 h. In the second phase, doses of 6000, 8000, 10,000, 12,000, and 14,000 mg/kg BW were administered to another set of animals. When no mortality was recorded, a third set of animals equally assigned to five groups were, respectively, treated with doses of 15,000, 20,000, 30,000, 40,000 and 45,000 mg/kg BW of the extract. They were closely observed for negative behavioral changes and mortality within 24 h of the experiment.

The lethal dose of the extract through the different routes was calculated using the formula by Lorke [12]:

$$LD_{50} = \sqrt{D_0 \times D_{100}}.$$

Where D_0 = Maximum dose that produces 0% mortality, D_{100} = Minimum dose that produces 100% mortality.

Indomethacin Induced Gastric Ulcer Model

A total of 30 adult male Wistar rats of weight between 130.30 and 163.00 g were randomly assigned to five groups as shown in Table 1. Groups I and II were administered saline, while Groups III, IV, and V were pretreated with cimetidine (100 mg/kg BW), ST (200 mg/kg BW), and ST (400 mg/kg BW) for 7 days, respectively. On the 8th day, gastric ulcer was induced by the method described by Bhattacharya *et al*. [14] with slight modification. Indomethacin (40 mg/kg BW) was administered to rats in Groups II, III, IV, and V after animals were fasted for 24 h. The animals were then sacrificed 4 h after the administration of indomethacin. The stomach of each animal was removed, incised along the greater curvature and the gastric content was emptied into appropriately labeled sample bottle for determination of gastric volume, pH, pepsin activity, free acidity, and total acidity. The stomach was then washed with normal saline, pinned on a flat surface to observe for lesions/ulcer in the glandular portion.

Quantification of Ulceration

The ulcerative index and percentage ulcer protection of the different treatments were estimated using the method described by Szabo and Hollander [15]. Based on the intensity of ulceration as observed with a hand lens, the score were given as: 0 = Normal

Table 1: Experimental design and treatment

Experimental group	Treatment/dose/route	Code
Group I	Rats were normal control	NC
Group II	Indomethacin rats were left untreated	UC
Group III	Ulcerated rats were treated with cimetidine (100 mg/kg BW/p.o)	PC
Group IV	Ulcerated rats were treated with plant extract (200 mg/kg BW/p.o)	ST 200
Group V	Ulcerated rats were treated with plant extract (400 mg/kg BW/p.o)	ST 400

NC: Normal control, UC: Untreated negative control, PC: Treated positive control, ST 200: 200 mg/kg BW/p.o ST extract, ST 400: 400 mg/kg BW/p.o ST extract, ST: *Sansevieria trifasciata*

mucosa; 1 = Vascular congestion; 2 = One or two lesions; 3 = Severe lesions, 4 = Very severe lesions, 5 = Mucosa full of lesions. The ulcer index was determined using the formula:

Ulcer index = (Ulcerated area/total stomach area) × 10

Percentage protection = (Uc−Ut) × 100/Uc

Where Uc = Ulcer index of control group and Ut = Ulcer index of treated group.

Estimation of Free Acidity

The gastric contents were centrifuged at 1000 rpm for 10 min. One mL of the supernatant liquid was pipette out and diluted to 10 mL with distilled water. The solution was titrated against 0.01 N NaOH using Topfer's reagent (dimethyl-amino-azo-benzene) as an indicator, to the end point when the solution turned to orange color. The volume of 0.01 N NaOH needed was taken as corresponding to the free acidity.

Estimation of Total Acidity

Titration was further continued by adding two drops of 1% solution of phenolphthalein till the solution gained the pink color. The volume of 0.01 N NaOH required was noted and was taken as corresponding to the total acidity.

Acidity was expressed as:

$$\text{Acidity} = \frac{\text{Volume of 0.01 N NaOH} \times \text{normality} \times 100 \text{ mEq/L/100 g}}{0.1}$$

Estimation of Pepsin Activity

The assay mixture contained 0.1 mL gastric juice supernatant (centrifuged at 5000 ×*g* for 10 min) and 1 mL of bovine albumin (0.5% w/v in 0.01 N HCl, pH 2) which was incubated for 20 min at 37°C. 2 mL of 10% trichloroacetic acid was added to stop the hydrolysis. All tubes were heated in boiling water for 5 min to denature the proteins and cooled. The precipitate was removed by centrifugation (9000 ×*g* for 10 min), and 1 mL of the supernatant was mixed with 0.4 mL of 2.5 N NaOH and 0.1 mL of the Folin–Ciocalteu reagent and the volume was make up to 10 mL with distilled water. A control set up in which 1 mL albumin was replaced with 1 mL of distilled water was run simultaneously. The absorbance was measured at 700 nm. The peptic activity was calculated in terms of micrograms of tyrosine liberated per milliliter of gastric juice according to the method described by Prino *et al.* [16].

Qualitative Phytochemical Evaluation of ST Extracts

Standard procedures as described by Sofowara [17]. Edeoga *et al.* [18], Trease and Evans [19] Harbone [20] were used with some modifications to detect the phytochemicals present in the extract of the plant.

Statistical Analysis of Data

The data obtained were statistically analyzed using GraphPad Prism statistical software, version 6.4. Hypothesis testing was by one-way analysis of variance followed by least significant difference test. $P < 0.05$ was considered statistically significant. Results are presented as mean ± standard deviation ($n = 5$).

RESULTS

LD_{50} Determination

i.p, the maximum dose of the extract that produced 0% mortality was 600 mg/kg BW and the minimum dose that produced 100% mortality was 1000 mg/kg BW.

The i.p LD_{50} of ST was estimated as 774.60 mg/kg, while the oral LD_{50} was estimated to be >18,000 mg/kg BW.

Antiulcerative Effects of ST

Compared to the control animals, single i.p administration of indomethacin at a dose of 40 mg/kg BW elicited a significant reduction in gastric juice volume, free acidity, total acidity, and pepsin activity as well as a concomitant increase in pH in experimental rats. However, oral pre-treatment of the animals with ST leaf extract at 200 and 400 mg/kg BW for a period of 7 days showed some significant effects in reducing the negative alterations caused by indomethacin in the animals [Table 2]. The extract was able to minimize the indomethacin-induced reduction in gastric volume (by 36.1%), free acidity (by 55.3%), and total acidity (by 35.6%) while minimizing the increase in pH (by 13.3%). Moreover, the extract showed 17.92% and 14.96 % ulcer protective ability at 200 and 400 mg/kg BW, respectively [Table 3]. This mild protection is substantiated by the photomicrographs shown in Figure 1 (a,b,c,d and e).

Qualitative Phytochemical Constituents of ST Leaf Extract

The phytochemical contained in ST ethanol leaf extract is shown in Table 4.

Table 2: Effect of ST extract on pH, free and total acid, and pepsin activity of the gastric juice in indomethacin-induced gastric ulcer in Wistar rats

Group	Gastric juice (ml)	pH	Free acidity (mEq/L)	Total acidity (mEq/L)	Pepsin activity (mg/mL)
NC	7.5±0.6	2.6±0.2	35.2±3.4	49.0±6.9	189.0±2.1
UC	3.6±0.4	5.6±0.4	9.6±0.6	20.8±2.5	131.0±33.6
PC	5.8±0.3*	3.5±0.5*	26.5±2.2*	37.3±3.0*	144.5±6.2*
ST 200	4.6±0.7	4.5±0.9	21.5±4.1*	30.7±3.2*	122.7±10.0
ST 400	4.9±0.5	3.9±0.3*	21.2±4.4*	32.3±4.1*	127.1±6.1

Values are expressed as mean±SD, n=5. *Significantly different (P<0.05) compared to indomethacin untreated group. SD: Standard deviation, NC: Normal control, UC: Untreated negative control, PC: Treated positive control, ST 200: 200 mg/kg BW/p.o ST extract, ST 400: 400 mg/kg BW/p.o ST extract, ST: *Sansevieria trifasciata*

DISCUSSION

Phytochemicals are chemical compounds formed during the plants' normal metabolic processes and have been associated with the biological or pharmacological effects elicited by plants. The medicinal functionality of several phytochemicals including alkaloids, flavonoids, coumarins, glycosides, phenols, tannins, terpenes, and terpenoids is well documented [21,22].

According to Martins *et al*. [23], the antitumor, anti-inflammatory, and antimicrobial properties of plant extracts are due to the presence of alkaloids. Flavonoids are known for their antioxidant characteristics [24]. Phenolics such as flavonoids and tannins have been linked with anti-helmenthic properties [25]. Saponins, in addition to their industrial uses such as foaming agents and detergents, have a wide range of medicinal applications [26].

In this study, the phytochemical screening of the ethanol leaf extract of ST shows that the plant constitutes most of these aforementioned biologically active phytochemicals, thus, signifying its use in folk medicine for treatment of different ailments including gastric ulcer. Moreover, the presence of these phytochemicals in ST suggests that the plant leaves possess valuable medicinal potential yet to be explored.

Despite the medicinal relevance of plants, studies have suggested that some plants or vegetable species are potentially toxic to humans and animals [27]. The chemical compounds responsible for the toxic effects of plants are probably produced as part of the plant's defense mechanism against pest and herbivores or to gain an advantage over competing plants. The toxicity status of a compound is often measured by its LD_{50}. LD or lethal concentration 50% is generally referred to as the dose or concentration of a test material (plant, chemical, drug, etc.) that causes mortality in 50% of the animals (rats, mice, etc.) in a dose group. LD_{50} values are useful in comparing the relative acute hazards of substances, especially when no other toxicology data are available for the substances. More importantly, the LD_{50} value of a material communicates its safety dose range through different routes.

Table 3: Inhibitory activity of ST leaf extract on indomethacin-induced ulcer in Wistar rats

Group (treatment)	Ulcer index (mean±SD)	Ulcer inhibition (%)
NC	0±0.0	-
UC	7.42±0.28[a]	-
PC	4.36±0.28[c]	41.24
ST 200	6.09±0.28[b]	17.92
ST 400	6.31±0.28[b]	14.96

Values with different superscripts are statistically significant ($P \leq 0.05$) to each other. SD: Standard deviation, NC: Normal control, UC: Untreated negative control, PC: Treated positive control, ST 200: 200 mg/kg BW/p.o ST extract, ST 400: 400 mg/kg BW/p.o ST extract, ST: *Sansevieria trifasciata*

Table 4: Phytochemical screening of ST

Phytochemicals	Result
Saponin	+
Flavonoid	+
Terpenoid	+
Cardiac glycoside	+
Alkaloid	+
Tannin	+
Anthraquinone	+
Glycoside	+

+: Present, ST: *Sansevieria trifasciata*

The LD_{50} values of the investigated leaf extract of ST through i.p route was 774.60 mg/kg BW. This value connotes a substantial degree of safety for the use of ST leaf extract in terms of toxicity level assessment through this route [28]. More importantly, oral administration of the extract at a dose of 18,000 mg/kg did not cause any negative behavioral changes in the animals, and no mortality was after 24 h of the experiment.

The strikingly high oral safe dose of ST could possibly be due to biotransformation of the active component(s) of the extract into nontoxic metabolites in the gastrointestinal tract of the animals by the action of certain modifying or detoxifying enzymes.

This study also evaluates the antiulcer activity of ethanol leaf extract of ST in indomethacin-induced ulcer model. Indomethacin is a NSAIDs which reduces pain, fever, and inflammation by inhibiting cyclooxygenase-1 (COX-1) and COX-2 (enzymes that produce prostaglandins, which promote pain, inflammation, and fever) [29]. COX-1 produces an additional type of prostaglandin that protects the stomach lining from stomach acid. Thus, inhibiting the enzyme makes the mucosal cells vulnerable to pepsin-acid damage, and consequently increases the risk of ulcers and gastrointestinal bleeding [30]. This is evident in the results of this study in which i.p administration of indomethacin at a dose of 40 mg/kg significantly altered valuable parameters associated with gastric secretion, and caused severe damage to the lining of the stomach [Figure 1a and 1b]. Nonetheless, oral pre-treatment of the animals with ST leaf extract at doses of 200 and 400 mg/kg BW for a period of 7 days significantly minimized the negative alterations caused by indomethacin in the experimental animals and showed some level of ulcer inhibition by the plant (17.92% and 14.96%, respectively) [Figure 1d and 1e]. The observed antiulcer activity of ST was however not comparable to that of cimetidine, a standard antiulcer drug, in that it was as much as 50% less effective than the drug [Figure 1c]. Nevertheless, the ulcer-inhibitory activity of ST in this study was estimated to be statistically significant relative to the ulcerated animals left untreated. Besides, the antiulcerative effect of cimetidine (41%) obtained in the study was below expectation, suggesting that the dosage of indomethacin used in this study may have caused severe gastric ulcer condition which appeared to have slightly subdued the potency of cimetidine. Anyways, considering the fact that the dosage employed (100 mg/kg BW) has been used in the previous studies [31,32] in which cimetidine was reported to offer better ulcer protection, it is, therefore, possible that the pharmacological response of the species of rats used in this study may have also played a significant role in the performance of the drug. In the same vein, any of these factors may have also

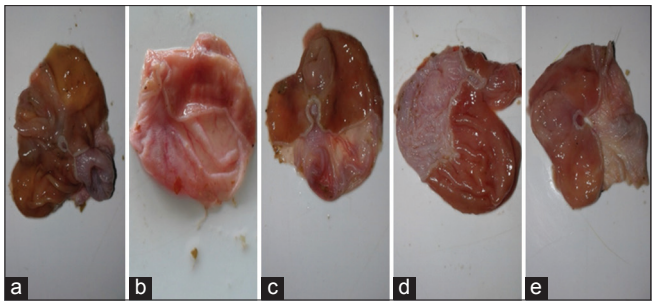

Figure 1: Photomicrographs of the stomach linings of indomethacin-exposed rats treated with cimetidine and *Sansevieria trifasciata* (ST) extract, (a) NC: Normal control, (b) UC: Untreated negative control, (c) PC: Treated positive control, (d) ST 200: 200 mg/kg BW/p.o ST extract, (e) ST 400: 400 mg/kg BW/p.o ST extract

affected the therapeutic performance of the plant (ST) extract. These postulations call for further investigations involving the use of a lesser dose of indomethacin/increased dose of ST on the antiulcerative potential of ST extract. Moreover, isolation and identification of the major antiulcerative compound in ST and subsequent evaluation of its antiulcerative potential will be necessary to arrive at a definite and meaningful conclusion on the effective use of ST as a remedy for gastric ulcer. Regardless, it is important to forecast the scientific basis for the antiulcerative potential of ST demonstrated in this study. The presence of antioxidant phytochemicals such as flavonoids and tannins in ST leaf extract may be partly accountable for its gastroprotective ability noted in this study. These compounds probably play a role in the metabolic activation of the COX enzymes that produce prostaglandins [29], particularly the COX-1 which produces an additional type of prostaglandin that protects the stomach lining from stomach acid and making the mucosal cells less vulnerable to hydrochloric acid and pepsin damage [30].

Although the indomethacin-induced increase in gastric pH noted in this study is at variance with the observation made in some previous studies [33,34], it may, however, be due to adaptive response of the experimental animals to the induced ulceration. In fact, it has been reported that the administration of indomethacin stimulates gastric bicarbonate secretion [35,36] which in turn can results in an increase in gastric pH.

CONCLUSION

The outcome of this study suggests that ST has a promising antiulcerative potential, along with herbal safety which is a major concern in the therapeutic utilization of plants. Further studies involving bioassay-guided identification of the main antiulcerative compound in ST is necessary to affirm and maximize the possible use of the plant as a therapeutic remedy for gastric ulcer.

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