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Study on acute ulcerous pain in rats treated with aqueous root extract of *Lonchocarpus cyanescens*

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ABSTRACT

Objective: To evaluate the antiulcer and analgesic effects of the aqueous root extract of *Lonchocarpus cyanescens* (*L. cyanescens*) since the plant is said to have medicinal properties.

Methods: The filtrate of the aqueous root extract of the plant (100 mg/mL) was used to evaluate for antiulcer activity in 20 rats divided into five groups of four rats each, which were Groups A, B, and C that received 100, 200 and 300 mg/kg doses respectively, while Group D was served as negative control and animals of Group E received 20 mg/kg dose of ranitidine. Indomethacin at a dose of 15 mg/kg was used to induce ulcer on the day of sacrifice. For acetic writhing test (antinociception), same design was used except that Group E received 100 mg/kg dose of acetyl salicylic acid as standard drug. Abdominal contractions were induced in the animals by intraperitoneal administration of 10 mL/kg of 0.6% of acetic acid.

Results: The aqueous root extract of *L. cyanescens* at all doses (100, 200 and 300 mg/kg) showed significant ($P < 0.05$) decrease in ulcer parameters compared with the negative control. The extract also produced a significant ($P < 0.05$) decrease in the number of writhing reflexes in treated rats compared with negative control.

Conclusions: The aqueous root extract of *L. cyanescens* exhibited both antiulcer and analgesic effects justifying folklore claim for the health benefits of this plant.

1. Introduction

Peptic ulcer, a gastrointestinal disorder, is usually acidic and extremely painful[1]. Peptic ulcers are small sores that form in the lining of the esophagus, stomach or duodenum[2]. Peptic ulcer disease can lead to serious complications including massive hemorrhage or bowel perforation[3]. The pathophysiology of peptic ulcer has to do with an imbalance between offensive factors such as acid, pepsin, or *Helicobacter pylori* and defensive counterparts including mucin, prostaglandin, bicarbonate, nitric oxide and growth factors[4]. It was thought that this imbalance is as a result of the association of several endogenous factors and aggressive exogenous factors leading to constant confrontation in the stomach and upper small bowel

between acid-pepsin aggression and mucosal defense. This imbalance is also known to release leukotrienes and reactive oxygen species[5]. It is said that factors such as alcohol consumption, use of steroidal and non-steroidal anti-inflammatory drugs, *Helicobacter pylori* infections, improper digestion, smoking, metabolism, elimination of food, mental and physical stressful lifestyle as well as drugs which stimulate gastric acid and pepsin secretion could contribute to the pathogenesis of gastritis[6]. Even though drugs are available for the treatment of this condition, indications are that the high incidences of side effects and drug interactions make these drugs of limited use. These side effects culminate in the search for the development of new antiulcer drugs in plants[7]. Medicinal plants have great applications in the African region, hence there is heavy reliance on these plants for alleviation of disease conditions in many rural areas of Africa[8–10]. There is therefore the need to evaluate some of these for efficacy in line with folklore's claims. In this study, *Lonchocarpus cyanescens* Benth (*L. cyanescens*) (Fabaceae) will be evaluated for its antiulcer and analgesic properties.

L. cyanescens is a shrub or tree that grows in savannah forest[11,12]. The bark is implicated in the treatment of bone pains, diabetes and the leaves and roots for treating boils and yaws[13].

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The entire herb of this plant is also said to have anti-inflammatory and anti-arthritic properties[14]. The leaves and roots of *L. cyanescens* are applied as a poultice to treat skin diseases, leprosy and ulcers but the roots are believed to be more effective than the leaves in curative effect. The decoction from the leaves and roots is also given to women during or after childbirth and this decoction may also used as an aphrodisiac. The decoction can also be used in the treatment of anti-arthritic conditions, venereal diseases and diarrhea[14]. Phytochemically, the leaves of *L. cyanescens* are rich in indoxyl which yields indigotin contained in the indigo dyestuff. Oleanane derivatives and glycyrrhetinic acid (GA) contained in this plant have anti-inflammatory properties and are responsible for relief of peptic ulcers observed in *L. cyanescens*[15]. The triterpenes act against arthritis[14–16].

2. Materials and methods

2.1. Plant collection and extract preparation

Fresh roots of *L. cyanescens* were collected at the University of Ibadan Campus and washed with water to remove the dirt. It was then authenticated at the Department of Botany University of Ibadan where a voucher specimen was deposited. About 20 g of the plant was macerated using mortar and pestle and then dissolved in 200 mL distilled water to make a 100 mg/mL concentration. This was then filtered using Whatman No. 1 filter paper and the filtrate collected was served as the aqueous extract used in this study.

2.2. Animals

A total of 40 healthy male albino rats weighing between 100 and 200 g used in this study were procured and kept at the Experimental Animal House, Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan and kept in iron cages and fed with standard diet and clean water *ad libitum*. All experimental procedures were conducted in accordance to the University of Ibadan Ethics Committee on Research in Animals. The study was conformed to internationally accepted principles for the use and care of laboratory animal[17].

2.3. Reagents and drugs

Drugs included indomethacin (Anthralon®, Ningbo Second Pharma, China), ranitidine tablets (Aciloc 150®, Cadila Pharmaceuticals, India) and acetylsalicylic and aspirin tablets (Bond Chemicals Co., Ltd). Normal saline and distilled water were also used.

2.4. The analgesic activity of L. cyanescens

A total of 20 male rats were randomly separated into five groups of four animals each and they were treated as follows: in Group I, rats were treated with the root extract of *L. cyanescens* at a dose of 100 mg/kg. Rats of Groups II and III were treated with 200 and 300 mg/kg root extract respectively. Rats of Group IV were served as the negative control group and received distilled water (2 mL/kg) while rats of Group V were served as the positive control group and received acetylsalicylic acid at a dose of 100 mg/kg. At 60 min after extract administration, abdominal contractions were induced in the animals by intra-peritoneal administration of 10 mL/kg of 0.6% of acetic acid[18]. The numbers of abdominal contractions over a period of 20 min following the injection of acetic acid were recorded. The degree of analgesia was calculated using the formula below:

$$\text{Degree of analgesia} = \frac{\text{Negative control}^* - \text{Treated group}^\#}{\text{Negative control}^*} \times 100$$

where, * was number of abdominal contraction of negative control and # was number of abdominal contraction of treated group.

2.5. Antiulcer study of L. cyanescens

The experimental rats were randomly separated into five groups of four rats each and were treated as follows: rats of Groups A, B and C were treated with *L. cyanescens* leaves extract at the dose of 100, 200 and 300 mg/kg respectively. While rats of Group D were served as the negative control group and received normal saline at a dose of 2 mL/kg, and rats of Group E were served as the positive control group and received ranitidine at 20 mg/kg. The plant extracts and drugs were administered to the animals for 8 day. After 8-day treatment, the animals were fasted for 24 h. Ulcer was induced using indomethacin at a dose of 15 mg/kg on the day of sacrifice. The animals were sacrificed at 6 h after indomethacin administration. After this the rats were eviscerated and the stomachs were removed and cut open along the greater curvature and washed in normal saline. Then it was laid flat and the number and degree of erosions were counted and scored[19]. The criteria were used in the scoring of the ulcer (Table 1).

2.5.1. Determination of preventive index

The preventive ulcer index was determined using the formula:

$$\text{Preventive index} = [(\text{Ulcer index of negative control} - \text{Ulcer index of treated group})/\text{Ulcer index of negative control}] \times 100$$

2.6. Statistical analysis

The results obtained in this study were expressed as mean ± SEM. The statistical analysis was performed using ANOVA. The student's *t*-test at 95% level of significance was used to assess significant difference between control and treated groups.

Table 1

The criteria of ulcer score.

Criteria	Ulcer score
No ulcer	0
Hemorrhagic and slight ulcer length less than 2 mm	1
Hemorrhagic and slight ulcer length less than 5 mm	2
More than 1 ulcer of grade 2	3
One ulcer less than 5 mm and diameter 2 mm	4
From 1 to 3 ulcers of grade 4	5
From 4 to 6 ulcers of grade 4	6
More than 6 ulcers of grade 4	7
Complete lesions with hemorrhage	8

3. Results

The administration of the aqueous root extract of *L. cyanescens* in acetic writhing produced a significant decrease ($P < 0.05$) in a number of writhing reflexes in treated rats at doses of 200 and 300 mg/kg only. While the dose of 200 mg/kg produced 18% decrease in writhing reflex and the dose of 300 mg/kg produced 43% decrease in writhing reflex indicating that dose-dependent decreased. It is to be noted that at dose of 100 mg/kg, there was no significant decrease ($P > 0.05$) in writhing reflexes in treated rats compared to negative control which indicated that at this dose, the analgesic effect of the root extract produced no effect (Table 2). In the case of the antiulcer activity, the aqueous extract of *L. cyanescens* roots at all doses (100, 200 and 300 mg/kg) showed significant ($P < 0.05$) decrease in ulcer parameters compared with the negative control. The dose of 200 mg/kg of the extract produced 80.7 preventive index being the highest by the doses of the extract. This was followed by the dose of 100 mg/kg at 71 preventive index. Incidentally, the dose of 300 mg/kg of the extract was the least at 64.5 preventive index. The result showed that there was dose related to the increase of preventive index between doses of 100 and 200 mg/kg but the decrease of preventive index at the dose of 300 mg/kg (Table 3).

Table 2

Effect of aqueous root extract of *L. cyanescens* on the acetic acid-induced abdominal contractions in rats ($n = 4$).

Groups	Writhing reflex	Pain inhibition (%)
I	52.8 ± 1.38[*]	0.00
II	43.0 ± 1.08[*]	17.78
III	29.8 ± 1.25[*]	43.02
IV (negative control)	52.3 ± 3.04[*]	0.00
V (positive control)	12.0 ± 0.71[*]	79.06

[*]: The values were significant ($P < 0.05$). Rats of Group I were treated with *L. cyanescens* root extract at a dose of 100 mg/kg. Rats of Group II were treated with *L. cyanescens* root extract at a dose of 200 mg/kg. Rats of Group III were treated with *L. cyanescens* root extract at a dose of 300 mg/kg. Rats of Group IV were negative control group and received 2 mL/kg distilled water. Rats of Group V were the positive control group and received acetylsalicylic acid at a dose of 100 mg/kg.

Table 3

Effect of aqueous extract of *L. cyanescens* roots on indomethacin-induced gastric ulcerations in rats.

Groups	Ulcer score	Preventive index
A	2.00 ± 0.42[*]	70.97
B	1.50 ± 0.50[*]	80.65
C	2.25 ± 0.48[*]	64.52
D (negative control)	7.75 ± 0.25[*]	0.00
E (positive control)	0.50 ± 0.29[*]	99.36

[*]: The values were significant ($P < 0.05$). A: Rats treated with *L. cyanescens* root extract at a dose of 100 mg/kg; B: Rats treated with *L. cyanescens* root extract at a dose of 200 mg/kg; C: Rats treated with *L. cyanescens* root extract at a dose of 300 mg/kg; D: Negative control group rats received normal saline at a dose of 2 mL/kg; E: Positive control group rats received ranitidine at 20 mg/kg.

4. Discussion

The extract of whole roots of *L. cyanescens* showed significant antinociceptive effect in acetic acid-induced writhing response. The writhe induced by acetic acid is a sensitive method deployed in assessing the analgesic effect of medicinal plants[19]. From the observation, it was concluded that aqueous extract of whole root of *L. cyanescens* at 200 mg/kg and 300 mg/kg body weight produced significant analgesic activity in albino rats while at the dose of 100 mg/kg, body weight did not produce significant difference as compared to control. However, the effect of aspirin (100 mg/kg), the non-steroidal anti-inflammatory drug used as positive control was greater than that observed for the extract. The significant analgesic activity of *L. cyanescens* may be due to its content of GA, a triterpenoid saponin which has been shown to possess anti-arthritic and anti-inflammatory activities[19]. Oleanolic acid and ursolic acid are triterpene saponins contained in the plant and may be responsible for the analgesic activity of the plant[20]. *Centella asiatica* whose major bioactive constituents are triterpene saponins which have also been reported to have analgesic and anti-inflammatory activity[21].

The antiulcer activity of the aqueous root extract of *L. cyanescens* was evaluated in rats using the indomethacin-induced ulcer model. A significant antiulcer activity was observed for the extract at all doses tested. Gastric mucosal damage caused by indomethacin and related non-steroidal anti-inflammatory drugs result from the inhibition of prostaglandins synthesis via the arachidonic pathway[21,22]. Thus, the effect of the extract in this model suggests that it may possess cytoprotective action probably by enhancing prostaglandins synthesis. *L. cyanescens* has been reported to contain GA, a genin of glycyrrhizinic acid which has been used extensively in the treatment of gastric ulcers[14,23]. GA is a triterpenoid saponin and this chemical class which has also been reported in licorice (*Glycyrrhiza glabra*) is known to offer protection against ulcers[7,24].

Ranitidine hydrochloride, the standard drug used in this study is a H_2-receptor antagonist. Its use as antiulcer drug is due to its ability to reduce acid secretion by blocking the histamine receptor type. Ranitidine is in the same class as cimetidine with the only difference that it contains furan ring in place of imidazole ring of cimetidine[25]. Any agents with anti-histaminic property may thus have some form of antiulcer property.

The plant under study, *L. cyanescens*, is rich in GA which inhibits the enzymes (15-hydroxyprostaglandin dehydrogenase and delta-13-prostaglandin) and that in turn metabolize the prostaglandins PGE-2α to their level of prostaglandins in the digestive system. Prostaglandin is known to inhibit gastric secretion but stimulate pancreatic secretion and mucous secretion in the intestines where it markedly increases the intestinal motility. GA, a kind of triterpenoid, has many pharmacological effects such as anti-inflammatory, antiviral, antiulcer, and adrenal cortical hormone kind function[7,14,26–29]. Clinical trials indicated that GA has a good therapeutic effect on different types of dermatitis, purulent scar disease, hair follicle infection and can as well as cure gingivitis, esophagus inflammatory disease. GA is also known to have anti-allergic, and anticancer activities[7]. GA has also been reported to exhibit selective toxicity to varieties of tumor cells, which makes it an ideal lead compound for anticancer treatment[30,31]. Histamine is released in inflammatory response and since GA has anti-inflammatory effect, this property may have exhibited through anti-histaminic effect hence its antiulcer effect may be similar to that of ranitidine[32]. This study may have thus justified the folkloric claim of this plant for analgesic and antiulcer activities.

Conflict of interest statement

The authors report no conflict of interest.

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