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Gastroprotective Activity and Potential Mechanism of the Stem Bark of *Boswellia dalzielii* on Gastric Ulcer in Rat

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Abstract: Gastric ulcer (GU) is the most common health concern that occurs due to alcohol consumption, smoking and physiological stress. *Boswelia dalzielii* (BD) is traditionally used to treat many diseases including gastric ulcers. This study aims to investigate the gastroprotective activity of *Boswelia dalzielii*. The cytoprotective property of *Boswelia dalzielii* was evaluated by using five experimental methods of GU: 1. Absolute ethanol, 2. HCl/Ethanol, 3. HCl/ Ethanol/ Indomethacin, 4. Indomethacin, 5. Pylorus ligation. Omeprazole was used as reference anti-ulcer drugs. Phytochemical screening of *Boswelia dalzielii* was carried out, and experimental Wistar rats (150-200 g) were used to evaluate the anti-ulcerogenic effects of the extract was carried out. Phytochemical screening of the effects of extract of *Boswelia dalzielii* extract revealed the presence of phytochemicals components such as tannins, saponins, flavonoids, triterpenes, and phenols. The oral administrations of 400 mg/kg of body weight of *Boswelia dalzielii* could protect the gastric mucosa against gastric ulcer. The results showed that *Boswelia dalzielii* at a concentration of 400 mg/kg has significant antiulcerogenic effects against absolute ethanol, hydrochloride ethanol (HCl/EtOH), HCl/ EtOH/IND, and pyloric ligation-induced gastric ulcer.

Keywords: Medicinal Plant, Gastric Ulcer, Gastroprotection, Pyloric Ligation

1. Introduction

The peptic ulcer is considered a worldwide chronic disease affecting millions of people and therefore associated with a higher rate of morbidity and mortality. Many factors are contributing to the development of peptic ulcer such as stress, dietary factors, high production of acid, helicobacter, pylori infection, and the use of nonsteroidal anti-inflammatory drugs [1]. Peptic ulcer results in damage of protective mucosal lining of the stomach and duodenum leading to the ulcerogenic

process [2]. The etiology of gastric ulcers is complex and multifactorial, mainly attributed to an imbalance between protective factors (mucus barrier, cytoprotective prostaglandins, antioxidants, bicarbonate secretion, and appropriate microcirculation) and harmful factors (highly acidic environment in the gastric lumen and pepsin activity) [3]. Treatment of gastric ulcers includes H_2- and M_1-blockers of histamine, proton pump inhibitors which decrease secretion of acid, and sucralfate and carbenoxolone which provide mucosal protection. Although these drugs have brought about remarkable changes in ulcer therapy, their efficacy is still debatable. Reports on clinical evaluation of these drugs show that there are incidences of adverse effects and drug interactions during ulcer therapy [4]. Therefore, an attention is paid to find more safe and potent nontoxic drugs. Among these new remedies are the herbal drugs due to their lower cost and side effects as well as easy availability [5].

Boswellia dalzielii is a plant that is popularly used for medicinal purposes in some African countries such as Ghana, Benin, Togo, Burkina Faso, Northern Nigeria, Cameroon, and Northern Ivory Coast [6, 7]. The plant is used by traditional healers to treat several diseases and illnesses which include digestive disorders, skin diseases, tuberculosis, nervous disorders, diarrheal disease, inflammation, analgesia, pyretic fever, and gingivitis [6, 7]. Experimental findings also established that the plant has antinociceptive properties [6]. The plant has many electron-rich compounds in abundance that can donate their electrons to reduce silver to synthesize silver nanoparticles [6]. This makes the plant a good candidate for nanoparticle production [8]. The electron-rich compounds the plant has include phenolics, flavonoids, alkaloids, glycosides, triterpenoids, carbohydrates, saponins, gallic acid, anthraquinones, protocatechuic acids, and β-sitosterol to mention but a few [8].

The objective of the present study was to investigate the antiulcer activities of the stem bark of the plant *B. dalzielii* in rats using.

2. Material and Methods

2.1. Plant Material and Preparation of Plant Extract

The stem bark of *B. dalzielii* was collected from the Beguele forest village in Maroua 2 subdivision in the far north region of Cameroon. The identity of the plant species was confirmed at the National Herbarium in Yaoundé, Cameroon, where a voucher sample was deposited under the registration number of 20532/SRF-CAM.

Four hundred grams (400 g) of powder was macerated in 3 liters of distilled water for 24 hours. After filtration using Whatman Paper No. 4, the solution was evaporated in an oven at 50°C, resulting in 18.6 g of extract (4.65% yield). The resulting extract was stored at 4°C for further use.

2.2. Phytochemical Screening

The sample powder was screened for the presence of biologically active compounds such as tannins, alkaloids, and glycosides, saponins, phenols and flavonoids, sterols, and triterpenoids. Qualitative assessment of the presence (+) of such phytochemicals was based on the intensity of coloration, the precipitate formed during the tests; their absence (-) was based on the absence of coloration or the precipitate during the tests [9].

2.3. Experimental Animals

Adult male Wistar rats weighing between 150-200 g were used in this study. Animals were maintained under standard conditions in the animal house of the Animal Physiology Laboratory, Faculty of Science, University of Yaoundé I. The animals were fed with all a standard laboratory diet and given freshwater ad libitum. All animal care and experimental procedures were carried in agreement with a protocol approved by the Cameroon National Ethics committee (Reg. No FWAIRB00001954).

2.4. Absolute Ethanol-Induced Gastric Lesions

The Absolute ethanol was used to induce ulcers in the gastric mucosa according to the method of Hara and Okabe [10]. Male rats were fasted for 36 h before administration of extract. The animals received the plant extract (100, 200 and 400 mg/kg) by oral route, 1 h before they were given the necrotizing solution. Positive and negative control rats received sucralfate and distilled water respectively in place of the extract. The animals were killed using ether, the abdomen of each opened and the stomachs removed. The ulcers produced in the glandular region of each stomach were measured and scored as earlier described by Tan et al. [11] and the ulcer index (UI), percentage of inhibition (% I) and percentage of ulcerated surface (%US) were calculated.

2.5. HCl/Ethanol-Induced Gastric Lesions

HCl/ethanol solution-induced lesions were provoked using the Absolute ethanol-induced gastric lesions method, by using HCl/ethanol solution. The rats received orally the plant extract (100, 200 and 400 mg/kg) or the vehicle after 1 h of HCl/ethanol treatment. They were also killed using ether and the lesions formed were observed and scored [10].

2.6. HCl/Ethanol-Induced Gastric Lesions in Rats Pre-Treated with Indomethacin

Indomethacin was given to the rats (20 mg/kg) by intra peritoneal route at the end of the 24 h fast. This was followed 1 h later by the HCl/ethanol ulcer procedure as described above [12].

2.7. Indomethacin-Induced Gastric Lesions

Twenty animals were depraved of food for 36 hours. Indomethacin (50 mg/kg) was given to the rats by oral route, 1 h after the animals received the vehicle, Sucralfate, and the extract (200 and 400 mg/kg). Five hours later, the animals were sacrificed under ethyl ether anesthesia [13] and the gastric lesions as well as the quantity of mucus were evaluated [11].

2.8. Pylorus-Ligated Gastric Secretion and Ulceration

The animals were fasted for 48 h with water ad libitum before pylorus ligation [9]. The rats in treatment group received the extract, while the controls received distilled water (1ml) or Omeprazole. One hour later, laparotomy was performed under ether anesthesia, the pylorus of each rat was ligatured, and the abdominal incisions stitched up. The gastric juice produced during six subsequent hours was collected from each rat and the volume was measured. The ulcers produced in the glandular region of the stomachs and quantity of mucus was measured. The percentage inhibition and the percentage of ulcerated surface were determined and considered as ulcer index.

2.9. Ulcerated Surface and Ulcer Index and Measurement of Mucus Contents and Gastric Juice Acidity

Ulcerated surface and ulcer index were calculated as described by Tan et al [11]. Ulcerated surface: length x width. Ulcer scores were allotted as follows: no ulcer = 0.0; ulcer surface $\leq 0.5mm^2$ =1; ulcer surface $>0.5 \leq 2.5mm^2$ = 2; ulcer surface $>2.5 \leq 5mm^2$ = 3; ulcer surface $>5 \leq 10mm^2$ = 4; ulcer surface $>10 \leq 15mm^2$ = 5; ulcer surface $>15 \leq 20mm^2$ = 6; ulcer surface $>20 \leq 25mm^2$ = 7; ulcer surface $>25 \leq 30mm^2$ = 8; ulcer surface $>30 \leq 35mm^2$ = 9; and ulcer surface $>35mm^2$ =10. The ulcer index (UI) was calculated with the following formula:

$$UI = \frac{1}{n}\sum_{1}^{n} \text{score} \pm \text{SEM}$$

The stomach of each rat was untied alongside the greater curvature. Then, the mucus covering of each stomach was gently scraped using a glass slide and the collected mucus was weighed carefully using a sensitive digital electronic balance [9]. The stomac contents were centrifuged were analyzed for hydrogen ion concentration by pH-metric titration with 0.1N NaOH solution. The acid contents were expressed as mEq/l.

2.10. Statistical Analysis

Data were presented as mean ±SEM. The data were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's post-hoc test using Graphpad Prism 9.50 Software. Values of $P<0.05$ were considered as statistically significant.

3. Results

3.1. Phytochemical Components

The results of preliminary phytochemical screening of the aqueous extract of *B. dalzielii* revealed that presence of flavonoids, tannins, terpenoids, Phenols and saponins.

3.2. Effect of B. dalzielii Extract on Absolute Ethanol-Induced Gastric Lesions

Table 1 shows the results obtained when the aqueous extract of *B. dalzielii* (100, 200 and 400 mg/kg) was used to prevent the formation of gastric lesions induced using absolute ethanol. As shown in Table 1, the ulcerated control rat group produced a low gastric mucus content. In contrast, animal groups treated with BD (400 mg/kg) exhibited significant increasing in the mucus weight (g) as compared to ulcer control rats with a significant high percentage of inhibition 50.37% inhibition against absolute ethanol-induced gastric lesions. Lesions provoked with absolute ethanol were located on the glandular part of stomachs and appeared like dark red bands (Figure 1). These bands were larger and more abundant in negative control (Photo a) just like in extract-treated (100mg/kg and 200mg/kg) rat (Photo b and c). Their sizes and numbers decreased in animals treated with BD (400 mg/kg) and positive control (Photo d and e).

Table 1. *Effect of B. dalzielii extract on gastric lesions induced by absolute ethanol.*

Treatment	Dose (mg/kg)	Ulcer index	% Ulcerated surface	% Inhibition	Mucus production (mg)
Control	-	5.33 ± 0.32	0.78	-	98.00 ± 9,02
B. dalzielii	100	4.55± 0.49	0.67	14.63	108.40 ± 10.96
B. dalzielii	200	4.26 ± 0.32	0.63	20.10	114.20 ± 18.37
B. dalzielii	400	2.64 ± 0.29***	0.39	50.37	133.60 ± 21.57**
Oméprazole	60	2.05 ± 0.31***	0.30	61.55	130.20 ± 10.07**

Values are represented as the mean ± Standard Error on Mean. ** $p < 0.01$ an d *** $p < 0.001$ indicate significant difference compared to control.

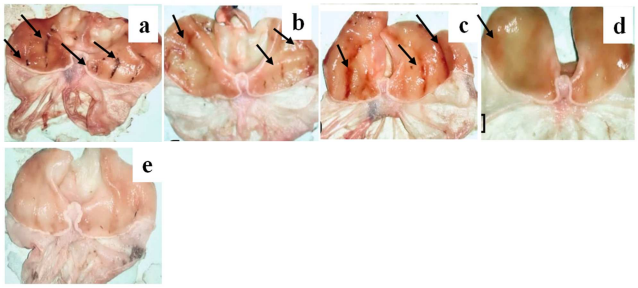

Figure 1. *Representative rat's stomach photomicrographs that were cut along the greater curvature. (a) Control, (b) BD 100 mg/kg, (c) BD 200 mg/kg, and (d) BD 400 mg/kg, (e) reference drug (omeprazole).*

3.3. Effect of B. dalzielii Extract on HCl/Ethanol-Induced Gastric Lesions

HCl/EtOH-induced severe mucosal injury in the rats without any treatment with total area of gastric lesion was 0.56% to the rats in control group (without gastric ulcer induction). Interestingly, the rats pretreated with BD at the dosage of 400 mg/kg total area of gastric lesion was significantly reduced compared to control group (Table 2). HCl/EtOH solution led to the formation of gastric ulcer, present as dark black color bands, on the glandular part of stomachs (Figure 2). These bands were numerous and larger in negative control (Photo a). Their number

and size dropped in positive control as well as in extract-treated rat (400mg/kg) (Photo d and e). This data indicated that only the high dosage of BD was able to suppress HCl/EtOH-induced gastric ulceration.

Table 2. *Effect of B. dalzielii extract on HCl/ethanol-induced gastric lesions in rats.*

Treatment	Dose (mg/kg)	Ulcer index	% Ulcerated surface	% Inhibition	Mucus production (mg)
Control	-	3. 81 ± 0.30	0.56	-	166.40± 10.14
B. dalzielii	100	3.64 ± 0.32	0.53	4.67	178.40 ± 21.15
B. dalzielii	200	3.51 ± 0.48	0.52	7.67	159.00 ± 16.25
B. dalzielii	400	1.86 ± 0.36**	0.26	51.18	207.00 ± 34.65**
Oméprazole	60	1.45 ± 0.45**	0.21	61.94	199.20 ± 17.09**

Values are represented as the mean ± Standard Error on Mean. ** $p < 0.01$ indicate significant difference compared to control.

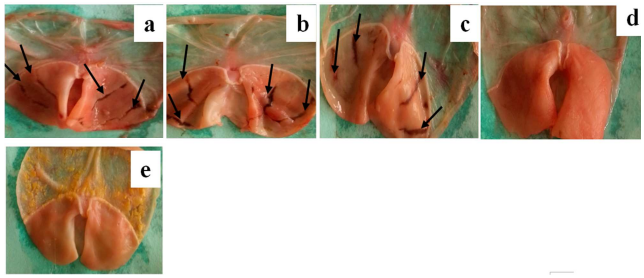

Figure 2. *Representative rat's stomach photomicrographs that were cut along the greater curvature. (a) Control, (b) BD 100 mg/kg, (c) BD 200 mg/kg, and (d) BD 400 mg/kg, (e) reference drug (omeprazole).*

3.4. Effect of B. dalzielii Extract on HCl/Ethanol-Induced Gastric Lesions in Rats Pre-Treated with Indomethacin

The effect of pre-treatment with indomethacin on the protective effect of the extract of BD against HCl/ethanol-induced lesions is shown in Table 2. This procedure had the effect of reducing the protective effect which the extract produced at the dose of 400 mg/kg. Thus, the prevention of lesion formation reduced from 51.18 % to 36.33% at 400 mg/kg extract due to indomethacin pre-treatment (Table 3). This was accompanied by a reduction in mucus production for all treated groups. These bands (Figure 3) were more abundant in negative controls (Photo a), their sizes and numbers decreased in animals treated with BD (400 mg/kg) (Photo c).

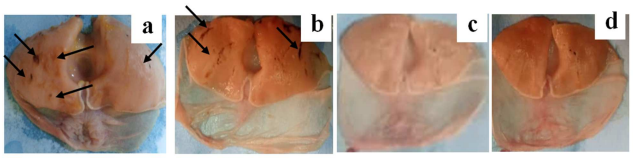

Figure 3. *Representative rat's stomach photomicrographs that were cut along the greater curvature. (a) Control, (b) BD 200 mg/kg, and (c) BD 400 mg/kg, (d) reference drug (omeprazole).*

Table 3. *Effect of B. dalzielii extract on HCl/ethanol-induced gastric lesions in rats pre-treated with indomethacin.*

Treatment	Dose (mg/kg)	Ulcer index	% Ulcerated surface	% Inhibition	Mucus production (mg)
Control	-	4.59 ± 0.27	0.71	-	75.5 ± 7.23
B. dalzielii	200	3.66 ± 0.19	0.46	20.26	95.2 ± 4.25*
B. dalzielii	400	3.05 ± 0.44**	0.44	36.33	121.0 ± 11.74 ***
Oméprazole	60	2.60 ± 0.29***	0.39	45.72	111.2 ± 9.22**

Values are represented as the mean ± Standard Error on Mean. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ indicate significant difference compared to control.

3.5. Effect of B. dalzielii Extract on Indomethacin-Induced Gastric Lesions

The aqueous extract of BD no showed a significant reduction in ulcer index and a non-significant increase in mucus content compared with controls when indomethacin was used as the necrotizing agent. However, inhibition of lesion formation was low significantly (14.97 and 14.29 %) for the doses of 200 and 400 mg/kg, respectively (Table 4). Gastric lesions obtained after induction by indomethacin are illustrated by (Figure 4). The lesions, appearing as small red or black bands, were more visible in negative control (Photo a).

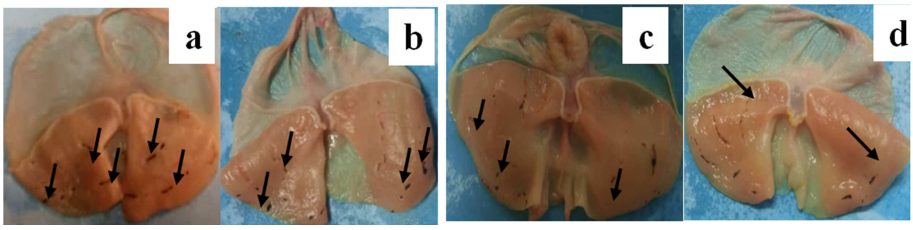

Figure 4. *Representative rat's stomach photomicrographs that were cut along the greater curvature. (a) Control, (b) BD 200 mg/kg, and (c) BD 400 mg/kg, (d) reference drug (omeprazole).*

Table 4. *Effect of B. dalzielii extract on indomethacin-induced gastric lesions in rats.*

Treatment	Dose (mg/kg)	Ulcer index	% Ulcerated surface	% Inhibition	Mucus production (mg)
Control	-	2.94 ± 0.04	0.44	-	46.0 ± 1.95
B dalzielii	200	2.50 ± 0.00	0.37	14.97	57.6 ± 1.12
B dalzielii	400	2.52 ± 0.02	0.38	14.29	56.8 ± 0.86
Omeprazole	60	1.29 ± 0.10**	0.19	56.12	56.2 ± 0.80

Values are represented as the mean ± Standard Error on Mean. ** $p < 0.01$ indicate significant difference compared to control.

3.6. Effect of B. dalzielii Extract on Pylorus-Ligated Gastric Secretion and Ulceration

As shown in Table 5 and Table 6, treatment with the extract and the standard drug reduced the volume of gastric secretion, both doses produced a statistically significant decrease in the total acidity when compared to the negative control, despite low percentage.

Table 5. *Effect of B. dalzielii extract on pylorus-ligated gastric ulceration in rats.*

Treatment	Dose (mg/kg)	Ulcer index	% Ulcerated surface	% Inhibition	Mucus production (mg)
Control	-	39.2±0,19	0.49	-	25.60±2.11
B dalzielii	200	2.73±0.19*	0.27	30.35	37.80±3.75
B dalzielii	400	2.67±0.27**	0.20	31.88	36.40±3.75
Omeprazole	60	1.93±0.06***	0.21	50.76	43.00±4.34**

Values are represented as the mean ± Standard Error on Mean. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ indicate significant difference compared to control.

Table 6. *Effect of B. dalzielii extract on gastric secretion in pylorus-ligated rats.*

Treatment	Dose (mg/kg)	Volume of gastric juice (mL)	pH of gastric juice	Gastric acidity (mEq/L)	% Reduction in gastric acidity
Control	-	6,84 ± 0,54	1,84 ± 0,1	65,00 ± 2,23	-
B. dalzielii	200	5.16 ± 0,67	2,04 ± 21	54,10±0,96***	16,77
B. dalzielii	400	5,80 ± 0,98	2,21 ± 0,23	57,40± 1,69**	11,69
Oméprazole	60	3,46 ± 0,73*	7,06 ± 0,26***	21 ± 0,41****	67,69

Values are represented as the mean ± Standard Error on Mean. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ indicate significant difference compared to control.

4. Discussion

Gastric ulcer is caused not only by over secretion of acid or pepsin, but also by reduced resistance of the stomach lining to gastric juices. Under normal conditions, the gastric mucosa is protected by bicarbonate-rich mucus that is secreted by the goblet cells or mucous cells which cover the entire luminal surface and extend down into the gland. Ethanol and ethanol/HCl are among the most utilized experimental models for the evaluation of antiulcer activity in rat [14]. These substances have been reported to be involved in the depression of gastric defensive mechanisms, the production of mucus and bicarbonate secretion, which contribute to the development of hemorrhagic and necrotic lesions [15]. The BD showed gastroprotective effect as evidenced by a marked inhibition at the 400 mg/kg dose on absolute ethanol- and HCL/ETOH-induced gastric lesions formation. The results of this study show that the extract of BD, when administered 1 hour before injury, protected the gastric mucosa by significantly increasing mucus production there by reducing lesion formation in both models at the dose of 400 mg/kg. The mucus covering of the entire gastrointestinal mucosa serves as the first line of the defense against physical damage and back diffusion of hydrogen ions. This mucus is a viscous, elastic adherent and transparent gel formed by water and glycoproteins. Its protective effects depend not only on the gel structure but also on the amount or thickness of the layer covering the mucosal surface. Thus, the ability of the gastric mucosa to resist injury caused by endogenous secretions (acid, pepsin and bile) and by ingested irritants such as alcohol, aspirin and NSAIDs can be attributed to a number of factors that have been generally referred to as mucosal defense [9].

HCl/EtOH-model with pre-treatment with the indomethacin was used to investigate the mechanism by which extract promotes mucus secretion. The pre-treatment with indomethacin exposes the gastric mucosa to damages caused by HCl/EtOH. Indomethacin, a nonsteroidal anti-inflammatory drug (NSAIDs), is commonly recommended in clinical practice because it reduces the inflammation by limiting the production of prostaglandins (PG) from arachidonic acids via inhibition of cyclooxygenase (COX). Changes in PG levels stimulate acid secretion, disrupting gastric homeostasis, increasing neutrophil infiltration, inducing tumor necrosis factor (TNF) expression, and disrupting the balance between free radicals and antioxidants [16]. Due to this, long-term or frequent usage of indomethacin is highly linked to a higher risk of adverse gastrointestinal events, including stomach mucosal erosion, ulceration, bleeding, and perforation [17]. The gastroprotective effects of endogenous prostaglandins on

gastric mucosa are well-known [16]. Indeed, prostaglandins are very important in protecting gastric epithelium against lesions by increasing mucus and bicarbonate production, enhancing the microcirculation in stomach, promoting the repair of gastric mucosa [17]. Thus, inhibition of prostaglandins predisposes the gastric mucosa to damages while its stimulation can be protective. In this study, BD provoked at 400mg/kg dose decrease of ulcerated surfaces (36.33% Inhibition) compared to negative control. This inhibition was associated to an augmentation of mucus secretion (121.0mg) comparatively to negative control (75.5mg). Indomethacin-induced ulcer model did not significantly reduce the damage of gastric mucosa. This result suggesting that its gastroprotective effect does not involves the increase in the prostaglandin synthesis but could act by a direct action on mucus secreting cells.

Pyloric ligation-induced ulcer model is an important method for the measurement of mean ulcer index in ulcerogenesis. Gastric ulceration in this method may be the stress induced secretion of HCl in excess amounts from the parietal cells and autodigestion of mucosa by the gastric juice [18, 19]. Free radicals may also be associated since studies have shown changes in the antioxidant status following pylorus ligation induced ulceration in rats [19]. In the present study, different doses of the extract (200, and 400 mg/kg) were evaluated for their effect on volume of gastric secretion, pH, total acidity, ulcer score, and ulcer index along with the standard drug omeprazole (60 mg/kg). This model showed that highest dose of the plant extract has antisecretory activity as evidenced by reduction in total acidity ($P<0.01$) compared to the negative control. Significant reduction in ulcer index (measure of ulcerated area) was noted for BD200 ($P<0.05$) and BD400 ($P<0.001$) as compared to the negative control. While omeprazole has more antisecretory effect than the extract. However, the mucus secretion from gastric wall was not significantly increased by BD treatment. We therefor suggest that BD inhibited gastric ulcer formation in the pyloric ligation model is likely associated with gastric acid suppression.

In the preliminary phytochemical screening, extract of BD was positive for flavonoids, saponins, tannins, phenols, and terpenoids. These secondary metabolites are effective as antioxidant, antineoplastic, anti-ulcer, anti-inflammatory, and immune stimulating agents [20]. Flavonoids are thought to increase mucosal prostaglandin content, decrease histamine secretion from mast cells by inhibition of histidine decarboxylase, inhibit Helicobacter pylori growth, act as free radical scavengers, and inhibit H+/K+-ATPase. Saponins may activate mucous membrane protective factors, and tannins render the outermost layer of the mucosa less permeable, for instance, to chemical irritation. In addition, terpenoids compounds are also reported to have potent activity against gastric ulcers [20]. The results suggest that the gastroprotective and antisecretory activity of the extract may be due to the combined effects of the different phytoconstituents present.

5. Conclusion

These findings findings clearly imply that BD at a concentration of 400 mg/kg has significant antiulcerogenic effects against absolute ethanol, hydrochloride ethanol (HCl/EtOH), HCl/ EtOH/NSAIDs, and pyloric ligation-induced gastric ulcer. This activity might be highly associated with mucus production, gastric acid suppression, antioxidant property of BD due to active compounds, such as flavonoids and phenols. Therefore, BD might be regarded as a promising source for the development of preventative and therapeutic agent for stomach ulcers.

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