Evaluation of the pharmacological interactions between coadministered *Newbouldia laevis* root bark extract and omeprazole in ulcer induced rat model

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Abstract
Ulcers are wounds on the lining of the gastrointestinal tract. There are different types of ulcers some of which include: duodenal, gastric and esophageal ulcers. The most common type of ulcers is peptic ulcers which occur mostly in those portions that come into contact with stomach acids and enzymes. Other causes of ulcers comprise antibiotics, anti-inflammatory drugs, alcohol and a microorganism called *Helicobacter pylori*. There are numerous antiulcer drugs in current use. These drugs are associated with many problems some of which are life-threatening. In other to avert these complications, herbal remedies are used recently because they are expected to be more efficacious and with high safety potentials. In this study, we evaluated the antiulcer interactions between coadministered *Newbouldia laevis* root bark extract and Omeprazole using rat model. The 12 mature Wister rats used in this study were divided into four groups of three rats each. Distilled water, Omeprazole, extract of *Newbouldia laevis* alone and coadministration of Omeprazole and *Newbouldia laevis* extract were given to groups I, II, III and IV respectively. After 30 minutes, ulcer was induced in all the rats using 95% ethanol. One hour later, all the rats were sacrificed and the gastrointestinal tract separated. The pH of gastrointestinal content was assessed and the number of ulcers counted. The least number of ulcers were observed in group IV. It was concluded that *Newbouldia laevis* and Omeprazole interacted synergistically because they, in combination, exhibited better ulcer protective effects than each of the treatments given alone.

Key words: Antiulcer, *Helicobacter pylori, Newbouldia laevis*, Omeprazole,

Running title: Interaction of *Newbouldia Laevis* and Omeprazole

Introduction

Ulcers are wounds on the lining of the gastrointestinal tract (GIT). There are different types of ulcers some of which include: duodenal, gastric and esophageal ulcers. The most common type of ulcers is peptic ulcers which occur mostly in those portions that come into contact with stomach acids and enzymes. Peptic ulcer disease (PUD) is the 10th cause of death therefore constituting a major health care problem (Wong *et al*., 2005; Tanih *et al*., 2010). The main risk factors for PUD are *H. pylori* and non-steroidal anti-inflammatory drugs (NSAIDs). *H. pylori* infects almost 50% of the global population (Papatheodoridis *et al*., 2005). NSAIDs are implicated in more than 90% of all ulcers and around 25% of NSAID users are at risk of developing peptic ulcer disease (Lanza *et al*., 2009). Other risk factors for peptic ulcer disease consist of ischemic heart disease, medications, viral infection, gastric bypass surgery, metabolic disturbances, radiotherapy, histamine, eosinophilic infiltration, and basophil (McColl *et al*., 2009). Research findings show that gastric ulcers are more likely to develop in older people. This may be because older people are more

prone to arthritis and in other to relieve arthritis pain, they resort to taking NSAIDs on daily basis. Some common symptoms of ulcer include: discomfort between meals or during the night as obtained in duodenal ulcer, discomfort when food or drink is ingested as in gastric ulcer, stomach pain, early-satiety, bloating, and burning sensation in the stomach. Lifestyles that play some roles in the development of ulcers include: smoking and alcohol intake (Narayanan *et al*., 2018). There are numerous antiulcer drugs in current use. These include: proton pump inhibitors such as omeprazole; selective Histamine Type 2 receptor antagonists such as cimetidine; antacids such as magnesium hydroxide; mucosal protective agents such as sucralfate and misoprostol; antibiotics such as clarithromycin. Treatment of ulcers involve combination of these drugs in triple or quadruple therapy (Malfertheiner *et al*., 2017; Strand *et al*., 2017; Xiao *et al*., 2013). These agents used for treating PUD have limitations such as generating other morbid states and causing adverse effects. In other to avert these complications and to provide a lasting solution, herbal remedies are used recently because they are expected to be more efficacious and with high safety potentials. Furthermore, more researches on these herbal drugs are coming up with the intention of discovering new approaches to ulcer treatment. Large numbers of medicinal plants and dietary nutrients which are safe with less adverse effects have been shown to exhibit gastroprotective activities (Sharma *et al*., 2011; Dharmani *et al*., 2005). Some examples of these medicinal plants are: *Syzgium aromaticum* which is also called clove (Okasha *et al*., 2007), *Cuphea eqipetala* leaves infusion (Palacios-Espinosa *et al*., 2014), *Newbouldia laevis* among others. *Newbouldia Laevis* is a medium sized angiosperm which belongs to the Bignoiaceae family and grows to a height of about 7-15 meters (Usman *et al*., 2007).The tree is commonly known as tree of life or boundary tree (Idu *et al*., 2009). It is called 'Aduruku', 'Adoko' and "Ogirisi' in Hausa, Yoruba and Igbo respectively. According to certain researches, *N. laevis* has medicinal values ranging from antiinflammatory, antioxidant, antimicrobial, antifungal, analgesic, and wound healing properties (Chukwujekwu *et al*., 2005; Kuete *et al*., 2007; Akerele *et al*., 2011). In the Southeastern part and the Midwestern area of Nigeria the *N. laevis* is used for the treatment of septic wounds (Akerele *et al*., 2011). It was also shown that *N. laevis* ethanol root bark extract had a significant dose dependent antiulcer effect which was attributed to its antioxidant, antiinflammatory, wound healing properties (Awemn *et al*., 2012). We therefore aim at evaluating the pharmacological interactions between *Newbouldia laevis* and omeprazole when administered concurrently.

Materials and Methods

Animals

Adult Wistar rats were bought from the animal house of Faculty of Pharmaceutical Sciences Nnamdi Azikiwe University Awka, Anambra State, Nigeria. The rats were acclimatized for one week and were fed with commercially available rat pellets and allowed accesses to drinking water ad libitum and maintained under laboratory conditions of temperature 26 ± 2 °C, humidity of 50 ± 5%, and at a 12 h natural light/dark cycle. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals and were approved by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes.

Chemicals and reagents

Methanol (Sigma-Aldrich**,** Bangalore, India), 95% Ethanol (Qingdao Hai Jier, Licang district, China), Omeprazole tablets (Wellona Pharma, Gujarat, India), 80% ketamine (Bnm Organics Pvt Ltd, M G Road, India).

Plant material

Newbouldia laevis root back extract (Faculty of Pharmaceutical Sciences, Awka, Nigeria)

Equipment

Water bath (Bioevopeak Inc, Seattle, USA), muslin cloth, No.1 whatman filter paper (Sigma-Aldrich Pty Ltd, Darmstadt, Germany), hand

gloves (HBM Health Protections, Inc, Guilin, China), Beakers (Hach, Dubai, United Arab Emirates), Measuring cylinders (Bellco Glass, Inc.,Vineland, US), Maceration bottle (Euroglass Hamburg, Germany), pH paper (Miotus Co., Ltd, Chongchuan, China), dissecting set (Servicebio technology Co., Ltd, Wuhan Hubei, China).

Methods

Preparation of *Newbouldia laevis* root bark extract

The roots of *Newbouldia laevis* were collected from a forest at Nise, Anambra state, washed and dried. After drying the root barks were detached and crushed. 200g of the powdered root bark was soaked in one liter of methanol for 48 hours. The filtrate was collected by sifting through a muslin cloth. The filtrate was concentrated in a water bath at 50^{o}C.

Phytochemical analysis of *N. laevis* root bark extract

Phytochemical tests of *N. laevis* root bark extract included tests for alkaloids, flavonoids, tannins, terpenoids, phenols, proteins, amino acids, carbohydrates and glycosides. These tests were carried out in accordance with the procedures described by Sahira and Cathrine (2015).

Tests for Alkaloids

A small number of drops of dilute hydrochloric acid was added to slight amount of the extracts samples, mixed, filtered and subjected to the following tests for alkaloids:

Mayer's reagent: A portion of the filtrates were treated with Mayer's reagent and observed. Occurrence of yellow or creamy precipitate indicates the presence of alkaloids.

Dragendoff's reagent: A portion of the filtrates were treated with Dragendoff's reagent and observed. Occurrence of reddish brown precipitate indicates the presence of alkaloids

Wagner's reagent: A portion of the filtrates were treated with Wagner's reagent and observed. Occurrence of reddish brown precipitate suggests the presence of alkaloids.

Hager's reagent: A portion of the filtrates were treated with Hager's reagent and observed. Manifestation of yellow precipitate indicates the presence of alkaloids.

Test for flavonoids

Lead acetate test: The extracts were treated with a few drops of lead acetate solution. Development of yellow precipitate indicates the presence of flavonoids.

Alkaline reagent test: The extracts were treated with a few drops of sodium hydroxide. Development of intense yellow color, which becomes colorless on addition of few drops of dilute acid, confirms the presence of flavonoids.

Test for reducing sugar (Carbohydrates)

Benedict test: Small quantities of the aqueous test samples were treated with Benedict solution and heated to boiling in water bath. Appearance of brick red precipitate indicated the presence of reducing sugar.

Fehling's test: Small quantities of the test samples in water were treated with equal volumes of Fehling's A and Fehling's B solution and heated in a water bath for 10 minutes. Development of red precipitate indicates the presence of a reducing sugar.

Test for Saponins

Frothing test: Extracts were treated with 20ml of water and shaken for about 15 minutes in a graduated cylinder. Formation of a stable foam layer specifies the presence of Saponins

Test for Proteins

Million's test: A 1 ml of test solutions each treated with sulphuric acid was added to a small amount of million's reagent and boiled. The sample was observed for formation of white precipitate which turns red after warming as an indication of the existence of protein.

Precipitation test: If the test solutions give white colloidal precipitate with 5% $CuSO_4$ and 5% Lead acetate, it is an indication of the presence of proteins.

Tests for Tannins

Ferric Chloride test: A 5% dilute ferric chloride solution was added to 2 ml of the extract. Violet color formation indicates the presence of tannins.

Test for Amino acids

Ninhydrin test: Three drops of 5% v/w lead acetate solution was added to 3 ml of the root bark extract, and heated to boiling in a water bath for 10 minutes. A change in color of the solution to purple or blue signified the presence of amino acids.

Test for Steroids and Triterpenoids

Salkowski test: Small amount of chloroform was added to 5 ml of the extract followed by the addition of few drops of concentrated sulphuric acid. The mixture was shaken well and kept aside for some time and observed. Appearance of red color indicated the presence of steroids and appearance of yellow color in the lower layer indicated the presence of triterpenoids.

Test for Glycosides

General test: This was carried out using the Fehling's method as explained for reducing sugar. After the Fehling's method, a portion of the red precipitate samples was hydrolyzed with dilute sulphuric acid in separate test tubes. Increase in intensity of the color indicates the presence of glycosides.

Acute toxicity studies (LD_{50}) of the root back extract of *Newbouldia laevis*

The median lethal dose (LD_{50}) estimation of the test drug was conducted using Lorkes method of 1983.

Evaluation of antiulcer activities

Experimental design

The 12 mature Wister rats used in this study were allocated into four groups of three rats each. These rats were starved for 24 hours preceding the administration of distilled water, omeprazole, leaf extract of *Newbouldia laevis* alone and co-administration of omeprazole and *N. laevis* leaf extract to groups I, II, III and IV respectively. The doses of each of these medications was determined by the weight of the rats and the results of the acute toxicity study of *N. laevis*. After 30 minutes of administration of these medications and distilled water, ulcer was induced in all the rats using 95% ethanol. One hour later, all the rats were sacrificed using 80% ketamine, dissected and the gastrointestinal tract (GIT) detached. The GIT content was evacuated into a bowl and set aside for evaluation of the effects of the medications on GIT pH values. The GIT was washed and observed for any ulcer that might have developed as a result of the 95% ethanol.

Results

Table 1: Results of phytochemical analysis of *Newbouldia laevis root back extract*

S/n	Phytochemical components	Tests	Methanolic extract of *N. laevis*
1	Flavonoids	Lead acetate	+
		Alkaline reagent	+
2	Alkaloids	Mayer's	+
		Dragendorf's	+
		Wagner's	+
		Hager's	+
3	Reducing sugars	Benedict's	-
		Fehling's	+
4	Saponins	Frothing	-
5	Proteins	Millions's	-
		Precipitation	+
6	Tanins	Ferric chloride	-
7	Amino acids	Ninhydrin	-
8	Steroids	Salkowski	-
9	Triterpenoids	Salkowski	-
10	Glycosides	General	-

The phytochemical component that occur most in N. laevis root back extract are flavonoids and alkaloids.

Results of acute toxicity studies of *Newbouldia laevis* metanolic root back extract

LD_{50} was > 5,000 mg/kg body weight.

Results of the antiulcer activities

Table 2: results for stomach content PH

Groups	Treatment administered	Mean PH ± SEM	p-value for pH	Mean ulcer number ± SEM (N)	p-value for N
I	Distilled water	7.0 ± 0.58	0.058	10.00 ± 4.36	0.036
II	Extract of Newbouldia laevis only	7.5 ± 0.29	0.036	6.00 ± 0.58	0.037
III	Omeprazole only	5.0 ± 0.00	0.025	13.00 ± 6.03	0.022
IV	Newbouldia laevis extract and omeprazole	6.5 ± 0.50	0.033	3.67 ± 0.33	0.028

Newbouldia laevis increased pH thereby reducing the acidity of the gastrointestinal tract while Omeprazole had the opposite effect. The two treatments given together interacted by potentiation.

The potentiation interaction between N. laevis and Omeprazole reduced the number of ulcers in group IV significantly.

Discussion

Phytochemical analysis of the root back extract of *Newbouldia laevis* showed the presence of flavonoids and alkaloids. This is in contrast with the work done earlier which reported that the phytocomponents of the leaves extract of *N. laevis* included Phenols, flavonoids, glycosides, tanins, oxalate, terpenoids, anthraquinolones, alkaloids and tanins which are present in both methanolic and aqueous extracts (Obum-Nnadi *et al*., 2020). A certain study reported that flavonoids represent a highly diverse class of secondary metabolites with potential beneficial effects on human health (Yao *et al*., 2004). According to the researchers, rhese compounds protect the gastrointestinal mucosa from lesions produced by various experimental ulcer risk factors and against different necrotic agents. Several mechanisms of action involved in this protective effect may include: anti-secretory mechanism, antihistaminic properties, inhibition of gastric H+/K+ proton pump (Kelly *et al*., 2009). Other mechanisms may comprise increase in mucosal blood flow which stimulate the synthesis of mucosubstances in the gastric mucosa and increase prostaglandins (PGs) levels, anti-*Helicobacter pylori* activity and most importantly antioxidant properties (Kelly *et al*., 2009). In another research, 61 alkaloids were reviewed and 55 of them reported to have antiulcer activities (Heloina *et al*., 2008). According to the results of acute toxicity studies, no death was recorded up to the administration of 5000 mg/kg body weight. This indicates that the stem back extract of *N. laevis* is safe and will not cause adverse reactions at the doses that will be recommended for treatment and prophylaxis of ulcer and its related illnesses. According to a recent study done on the leaf and root extracts of *N. laevis*, these extracts may not be as toxic as many medicinal plants, especially when used at doses not greater than 400 mg/kg body weight (Agbafor *et al*., 2015). The distilled water liquid (DWL) extract may be hepatoprotective at doses not greater than 400 mg/kg body weight (Agbafor *et al*., 2015). With respect to stomach pH and in comparison with the group I which is the control group, *N. laevis* decreased the acidity of the stomach by increasing the pH of group II rats significantly ($p = 0.036$) to 7.5 as compared to pH of 7 in group I. This contributed to the significant decrease in number of ulcers observed in the group from 10 to six. In another study, ethanol extract of *Newbouldia laevis* root bark had significant ($P < 0.05$) and dose dependent wound healing and antiulcer effects (Awemu *et al.,* 2012). The researchers noted that the percentage wound healing on day 14 of the study at 50, 100 and 200 mg/kg body weight of the extract were 62, 82 and 95% respectively. Both the wound healing and ulcer protection were comparable with those of standard drugs (Awemu *et al.,* 2012). The antiulcer property of the crude extract and fractions of the plant was investigated against ethanol induced gastric ulcer in white albino rats using Cimetidine (100 mg/kg body weight) as the standard control. The researchers concluded that the extract and fractions of *N. laevis* possesses significant antiulcer activity in animal models (Ogechukwu *et al.,* 2015).. The gastric antisecretory and acid neutralizing effect of the plant plus its antibacterial activity reveals the antiulcer potential of the plant (Ogechukwu *et al.,* 2015). On the other hand, omeprazole increased the acidity of the stomach significantly and decreased the pH to 5. Consequently, it increased the number of ulcers to 13 which is more than the number obtained in the control group. This is unexpected of a proton pump inhibitor such as omeprazole. However, the increased acidity maybe due to the fact that omeprazole is not a direct antacid. It might not have been able to inhibit acutely induced acidity. This is evident in a study which reported that in long term prevention studies, omeprazole (20 mg daily) and pantoprazole (40 mg daily) were shown to reduce the risk of gastric and duodenal ulcers and none steroidal anti-inflammatory drug related dyspepsia (Lazzaroni *et al*., 2001). Interestingly, when given together with omeprazole in group IV, *N. laevis* counteracted

the inability of omeprazole to decrease acutely induced acidity leading to a significant reduction in the number of ulcers to a value of 3.67, much lower than that obtained in the control group. This might be attributed to herb-drug interaction. *Newbouldia laevis* is a potent inhibitor of CYP1A2, CYP2C9, and CYP2C19 enzyme activities with Ki of 2.84 µg/mL, 1.55 µg/mL, and 1.23 µg/mL, respectively (Thomford *et al*., 2016). According to the researchers, the observations suggest the potential for herbal compounds such as *Newbouldia laevis* to interact, especially when coadministered with other medications metabolized by these CYP450 enzymes. Omeprazole is metabolized by CYP2C subfamily (Petersen *et al*., 1993). Consequently, inhibition of these isozymes by *Newbouldia laevis* prolonged the duration of action of omeprazole.

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Conflict of Interest
The authors declare no conflict of interest

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