ANTI-ULCERATION, ANTI-DIARRHEAL AND ANTI-ENTEROPOOLING POTENTIAL OF METHANOL EXTRACT OF ROOT OF *Napoleona imperialis* IN ALBINO RATS

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ABSTRACT

Anti-ulceration, anti-diarrheal and anti-enteropooling potentials of methanol extract of root of *Napoleona imperialis* in albino rats were investigated.60 albino rats weighing between 100 – 180 g used for the study were divided into five groups (Groups 1 – 5) with twelve animals in each group. Ethanol and indomethacin were used to induce gastric ulceration while castor oil was used to induce diarrhea and enteropooling in respective models which consisted of three animals per model per group. Group 1 served as positive control for each model while animals in Group 2 received standard drug for management of each of the conditions (cimetidine and propanolol for ulcer, loperamide for diarrhea). Group 3, 4 and 5 were administered 200, 400 and 600 mg/kg per body weight of the extract. Administration of the drugs to Group 2 and the extract to Groups 3, 4, and 5 was done one hour before the induction of respective conditions. Thirty minutes after the induction, the models for enteropooling study were sacrificed and intestinal fluid content measured. Diarrhea induced models were observed for consistency of fecal matter and the frequency of defecation for three (3) hours. The ulcer induced models were sacrificed and the stomach excised and observed microscopically for ulcerations. The result shows a dose dependent significant reduction of ulcer index in the indomethacin induced models administered with the extract when compared to control. Dose dependent reductions in consistency of fecal matter, frequency of defecation and intestinal fluid content were observed in diarrhea induced models and enteropooling effect. This result suggests that methanolic root bark extract of *N. imperialis* exhibit antiulcer, anti-enteropooling and antidiarrheal potential.

Keywords: *Napoleona imperialis*, Diarrhea, Ulcer, Enteropooling and Ulcer.

INTRODUCTION

Diarrhea is a gastrointestinal disorder, characterized by an increase in stool frequency and changed consistence. It is defined by the World Health Organization as having three or more loose or liquid stools per day, or as having more stools than is normal for that person (WHO, 2013) while acute diarrhea is defined as an abnormally frequent discharge of semisolid or fluid fecal matter from the bowel, lasting less than 14 days (WGO, 2011). Diarrhea may be classified as secretory (Jay *et al*., 2015), osmotic, inflammatory and exudative (David, 2011). It accounts for more than 5 - 8 million deaths each year in mostly children under five years old, especially in developing countries (Victoria *et al*., 2000).

In many cases of diarrhea, replacing lost fluid and salts is the only treatment needed. This is usually by mouth – oral rehydration therapy – or, in severe cases, intravenously. Diet restrictions are no longer recommended (King *et al*., 2003). Medications such as loperamide (Imodium) and bismuth subsalicylate may be beneficial; however they may be

contraindicated in certain situations (Schiller, 2007). To combat this problem, the World Health Organization (WHO) has initiated a diarrhea disease control program to study traditional medicine practices and other related aspects, together with evaluation of health education and prevention approaches (Damiki, 2011; WHO, 2004).

Stomach ulcer or ulcer in general is a gastrointestinal disease caused by a gram negative, spiral and flagellated bacterium called *Helicobacter pylori*. It is a major etiological factor in chronic gastritis, peptic ulcer, gastric carcinoma gastric mucosal associated lymphoid tissue (MALT) lymphoma (Suerbaum and Michetti, 2002; Oluwasola et al., 2002). The prevalence of this bacteria and hence the disease in Nigeria has been studied and is reported to be high (Jemilohun et al., 2010; Oluwasola et al., 2002; Obiajuru and Adogu, 2013; Tijjani and Umar, 2008; Nwokediuko et al., 2012). Few conventional therapies exist for the management of the condition, however, there is increasing interest in the use of herbal medicine hence the need for investigation into plants with curative or prophylactic potentials.

Napoleona imperialis, (family lecythidaceae) is an indigenous plant used in some Nigerian communities for the treatment of diarrhea, and bacterial infections. The bark and fruit pulp are chewed to alleviate pulmonary problems. Its wound healing (Esimone *et al*., 2005), anti-hypertensive (Omale *et al*., 2011), anti-plasmodial (Ogbuehi *et al*., 2014), and antimicrobial properties (Onyegbule *et al*., 2011) has been demonstrated. Its leaf and root extract contains glycosides, tannins, proteins and saponins (Omale *et al*., 2011). Extracts from the leaves and toxic seeds display bactericidal activity and contain various glycosides, tannins, proteins and saponins, while flavonoids, resins and steroids are absent. (Chukwu *et al*., 2014). In this study, we sought for justification for the folkloric use of the root of *N. imperialis* as an antidiarrheal agent as well as management of gastric ulcer using albino Wistar rat model.

MATERIALS AND METHODS

Plant material and Extract preparation

Roots of *N. imperialis* was gotten from Mgbirichi community in Imo State and were identified at the herbarium, University of Nigeria, Nsukka. The roots were washed clean with water then air-dried at room temperature and reduced to fine powder by milling. The powdered plant materials were subjected to extraction with 80% methanol for 48 hours. The hydromethanolic extracts were concentrated using a rotary evaporator (Rotavapor R- 200, Büchi) and allowed to paste using a water bath set at 40°C and stored at 4°C until used.

Induction of Diarrhea and Enteropooling in Albino Wistar Rats

Diarrhea and enteropooling was induced in the experimental animals by the administration of 2 ml of castor oil to each animal. Castor oil is a triglyceride characterized by high content of unsaturated fatty acid ricinoleic acid. About 90% of ricinoleate present in castor oil is mainly responsible for diarrhea production (Mckeon *et al*., 1994). After oral digestion of the castor oil, ricinoleic acid is released by lipases in the intestinal lumen, and considerable amounts of ricinoleic acid are absorbed in the intestine. This results in irritation to the intestinal mucosa and elicit inflammation, which releases prostaglandins and nitric oxide, responsible for the stimulation of gastrointestinal secretion, motility, epithelial permeability (Emmanuel *et al*., 2011), and edema in the intestinal mucosa, thereby preventing the reabsorption of Na^+, K^+ and water.

Induction of Gastric Ulceration in Albino Wistar Rats

Indomethacin and ethanol at 60 mg/kg and 2.5 ml respectively were used to induce gastric ulcer in the animals according to the methodology described by Okokon et al., (2011).

Experimental Design

A total of 60 albino Wistar rats weighing between 100 – 180 g were used for the study. They were divided into five groups (Groups 1 – 5) of twelve animals per group and were maintained under standard laboratory conditions, given access to standard diet and water *ad libitum*. The animals were used in accordance with the NIH (National Institution of Health) guide for the care and use of Laboratory Animal. Prior to the start of the experiment, the animals were fasted for 24 hours while water was withdrawn for two hours.

The first batch of three animals in each group were indomethacin-induced ulcer models. Group 1 (control) received only indomethacin (sigma, 60 mg/kg/bw) while Group 2 received the reference drug cimetidine (400 mg/kg/bw) and Groups 3 – 5 were pretreated orally with 200, 400 and 600 mg/kg/bw of root extract respectively. One hour later Groups 2 – 5 were administered 60 mg/kg/bw of indomethacin. Four hours after, the entire animals were anaesthetized with chloroform and dissected. The stomachs were removed and opened along a greater curvature. The tissues were fixed with buffered formaldehyde for macroscopic examination using hand lens and the presence of lesions was scored.

Similarly, the second batch of three animals in each group were induced with ulcer using ethanol and treated as follows; Group 1 (control) received only ethanol (2.5 ml/kg/bw) while Group 2 received the reference drug propranolol (40 mg/kg/bw) followed by 2.5 ml/kg/bw of ethanol one hour later. Groups 3 – 5 were pretreated orally with 200, 400, 600 mg/kg/bw of root extract of *Napoleona imperalis* respectively then followed by 2.5 ml/kg/bw of ethanol One hour later. Three hours after ethanol administration, the animals were anaesthetized with chloroform and dissected. The stomachs were removed and opened along a greater curvature. The tissues were fixed with buffered formaldehyde for macroscopic examination using hand lens and the presence of lesions was scored.

Furthermore, the third batch of three animals from each group were induced with enteropooling using castor oil and treated as follows; Group 1 serves as the control and received 5 ml/kg of water, Group 2 was given a standard drug (loparamide, 3mg/kg/bw), while Groups 3, 4 and 5 received orally, 200, 400 and 600 mg/kg/bw of the extract respectively. One hour after treatment with the extract, the animals were challenged with 2.0 ml of castor oil orally. After thirty minutes of castor oil administration, they were sacrificed, the small intestines were ligated at both pyloric sphincter and at the ileocecal junctions. The entire length of the small intestine was dissected out, and its contents was expelled into a graduated measuring cylinder and the volume measured.

Finally, the fourth batch of three animals from each group were induced with diarrhea using castor oil and treated as follows; Group 1 serves as the control and received 5ml/kg of water, Group 2 was given a standard drug (loparamide, 3mg/kg/bw), while Groups 3, 4 and 5 received orally, 200, 400 and 600 mg/kg/bw of the extract respectively. The animals were then housed singly in cages lined with transparent paper. One hour after treatment with the extract, the animals were challenged with 2.0 ml of castor oil orally. They were observed for

consistency of fecal matter and the frequency of defecation for three (3) hours. The wet fecal matter was read.

Statistical analysis

Data were presented as mean ± SEM. The significance of difference between the control and treated groups was determined using graph pad prism analysis of variance (ANOVA), followed by Student's *t*-test. *P* value of 0.05 or 0.01 was considered as significant.

RESULTS

Effects of *Napoleona imperalis* Root Extract on Indomethacin Induced Ulceration:

Qualitative analysis presented in Table 1 compares cimetidine to the extract of root of *Napoleona imperalis*. The result shows that there is significant difference ($P < 0.05$) when Groups 2 – 5 were compared to the control (Group 1). The rat treated with the plant extract shows significant dose dependent reduced ulceration with increasing dose when compared to control. However, cimetidine treated group showed a better ulcer index as well as preventive ratio.

TABLE 1: Effects of *Napoleona imperalis* Root Extract on Indomethacin Induced Ulceration

Treatment	Ulcer index	Preventive ratio
Group 1 received 5 mg/kg of H_20	3.000 ± 0.000	_____
Group 2 received 400 mg/kg of cimetidine	0.167 ± 0.167*	94. 43
Group 3 received 200 mg/kg *N.imperalis*	1.333 ± 0.333*	55.57
Group 4 received 400 mg/kg of *N.imperalis*	1.000 ± 0.500*	66.67
Group 5 received 600 mg/kg of *N.imperalis*	0.333 ± 0.167*	88.90

Data presented as Mean ± SEM
* significantly different when compared to control at $P < 0.05$

Effects of *Napoleona imperalis* Root Extract on Ethanol induced ulceration:

The effect of root extract of *Napoleona imperalis* on ethanol induced ulceration in albino Wistar rats is presented in Table 2. A significant reduction in the ulcer index is recorded for Group 2 when propranolol was administered before ulcer induction. The extract did not have any effect on the ulcer index at dose of 200 mg/kg/bw when compare to the control likewise the reduction of ulcer index at 400 mg/kg/bw of the extract. However, a significant reduction of ulcer index was observed at dose of 600 mg/kg/bw of the extract.

TABLE 2: Effect of *Napoleona imperalis* on Ethanol induced ulceration

Treatment	Ulcer index	Preventive ratio
Group 1 received 5 mg/kg of H_20	3.667 ± 1.333	_____
Group 2 received 40 mg/kg propranolol	0.333 ± 0.333*	90.92
Group 3 received 200 mg/kg *N.imperalis*	3.667 ± 0.333	0.00
Group 4 received 400 mg/kg *N.imperalis*	2.333 ± 0.333	36.38
Group 5 received 600 mg/kg *N.imperalis*	0.667 ± 0.333*	81.81

Data presented as Mean ± SEM
* significantly different when compared to control at $P < 0.05$

Effects of *Napoleona imperalis* Root Extract on Castor Oil-Induced Enteropooling

N. imperialis was found to have anti-enteropooling activity. The extract showed a dose dependent decrease in fluid accumulation which was significant at the highest dose, 600 mg/kg/bw when compared to the control. Figure 1.0 shows the anti-enteropooling effect of *N. imperialis* in rats administered with castor oil.

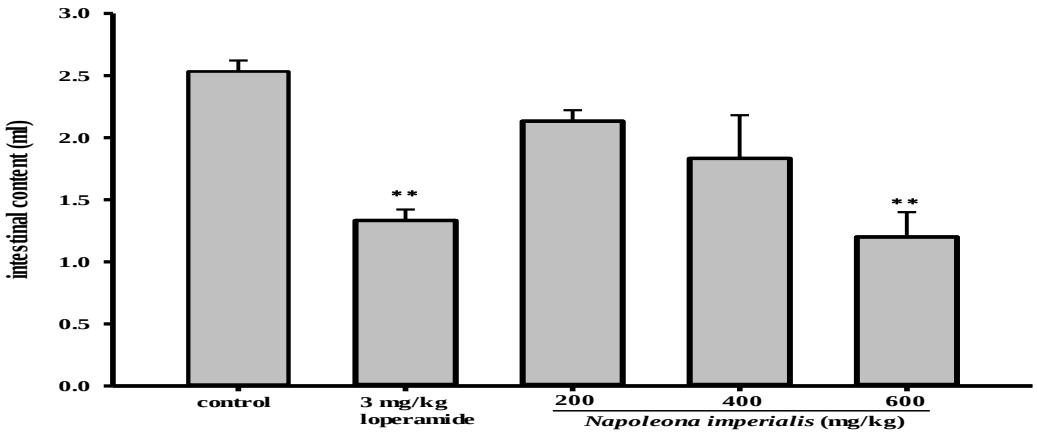

Figure 1.0: Effect of *N. imperialis* on Castor oil-induced enteropooling (intestinal fluid content). ** significantly different from the control at $P < 0.05$

Effects of *Napoleona imperalis* Root Extract on Castor Oil Induced Diarrhea

The *N. imperialis* methanolic root extract showed a considerable anti-diarrheal effect in rat. At doses of 200, 400, 600 mg/kg/bw, the extract caused a significant dose dependent decrease in the total number of wet feaces produced upon administration of castor oil when compared to control group. However, the highest dose of the extract, 600mg/kg produced a level of inhibition that was slightly less than that of a standard anti-diarrhea drug loparamide. Castor oil increased the number of wet and dry feces as well as weight of solid and wet feces. Methanolic root extract of *N. imperialis* exhibited a significant inhibition of castor oil-induced diarrhea in a dose dependent manner and the extract led to a marked reduction in total intestinal content also in a dose dependent manner.

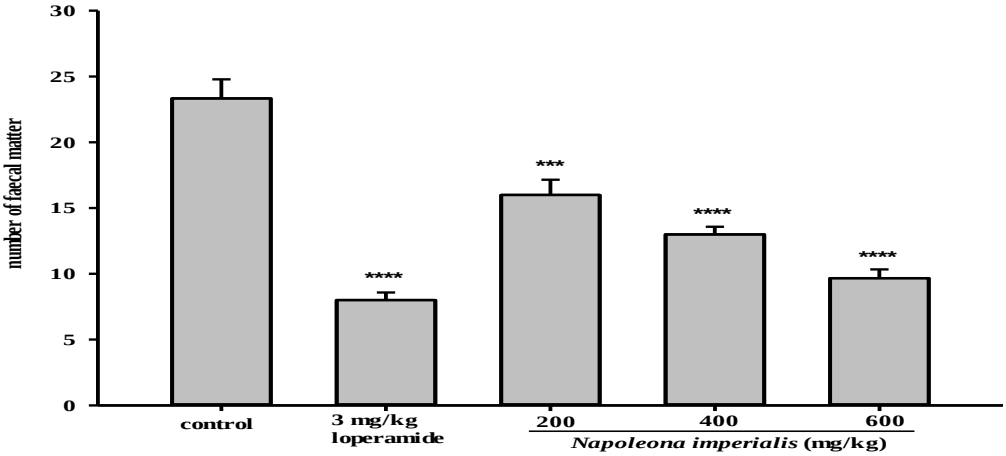

Figure 2.0: Effect of *Napoleoona imperialis* on Castor oil-induces diarrhea (fecal count).

DISCUSSION

The morbidity and mortality associated with diarrhea underscores the need for its effective control (Victoria *et al*., 2000). Due to problems such as malnutrition, diminished growth, impaired cognitive development and death caused by diarrhoea, there is need for alternative measures that will drive the achievement of the Millennium Development Goals. One of the alternatives may lie within the highly patronized plant and plant products used as medicines in many developing countries (Ezekwesili *et al*., 2010). In addition, plants hold promise as sources of new drugs. In the light of this, we investigated the antidiarrheal potential of methanolic leaf extract of *Napoleona imperialis*.

Loperamide is an opioid receptor agonist and acts on the μ-opioid receptor in the mysenteric plexus in the large intestine, but it does not affect the central nervous system like opioids, (Fauci *et al*., 2005). Loparamide acts by decreasing the transient velocity and increasing the capacity of the intestine to retain their fluid. (Teke *et al*., 2007). According to Ihekwereme *et al* (2016) it indicates that *N. imperialis* extract possibly reduces diarrhoea by increasing the reabsorbsion of electrolytes and water or by inhibiting induced intestinal accumulation of fluid just like loparamide.

N. imperialis extract reduced fecal matter and caused a marked reduction in the volume of intestinal content. Reduction of intestinal volume is often due to increased reabsorption of water, sodium, and potassium ion concentration. The sodium and potassium transport in the intestine has been related to membrane bound enzyme sodium and potassium. In diarrhea conditions, the decrease in Na^+ and K^+ ATPase occurs relating to an interception in the normal water and electrolyte absorption. Therefore, the reduction of water together with Na^+ accumulation might have an effect on the activity of Na^+ and K^+ ATPase. Hence, *N. imperialis* extract appears to stimulate the reabsorption of intestinal fluids in the intestine. Plant extracts containing tannin, flavonoids, alkaloids, saponins and steroids have been reported to possess anti-diarrheal activity (Shemsu *et al.*,2013; Balaji *et al.*,2012).

N. imperialis has been observed to contain Procyanidins, ellagic acid, pimoresinol, isoprunetin (Ihekwereme *et al*.,2016). Procyanidins and ellagic acid may be responsible for the observed antidiarrheal property since previous studies in other plants have identified them to be responsible for such activities (Hai-Tao Xiaoa, et al, 2013). Procyanidins are condensed tannins while ellagic acid is the dilactone of hexahydroxydiphenic acid. Both compounds are natural polyphenolic compounds. Plants produce ellagic acid from hydrolysis of tannins such as ellagitannin and geraniin. Raji *et al*. (2001) reported that methanol extract of the stem bark of Irvingia gabonensis which possesses 2,3,8-Tri-O-methyl ellagic acid has antidiarrheal properties in rats. Furthermore, Derivatives of pinoresinol and isoprunetin are not known for possessing antidiarrheal property. Consequently, it is reasonable to suspect that the procyanidins and ellagic acid derivatives present in *N. imperialis* may be responsible, either partly or wholly for the antidiarrheal property observed.

Indomethacin is a known ulcerogen especially in stomach, it is known to cause ulcer usually in an empty stomach and mostly on the glandular (mucosal) part of the stomach (Nwafor *et al*., 1996) by inhibiting prostaglandin synthetase through the cyclooxygenase pathway. Administration of ethanol has been reported to cause disturbances in the gastric secretion/damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production. This is attributed to the release of superoxide anion and hydroperoxy

free radicals during metabolism of ethanol as oxygen derived free radicals has been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa.

In this study, a dose dependent reduction in the ulcer index was observed in the rats with indomethacin induced ulceration following administration of the extract. However, the group treated with the standard drug cimetidine showed better protective effect against induction of the ulceration. However, a significant effect of the extract on ethanol induced ulcer model was observed only at high dose of 600 mg/kg/bw. The standard drug propranolol still had a more superior effect though not significantly different from the high dose of the extract. The results suggest a protective effect of the extract against gastrointestinal ulceration.

This may be due to cytoprotective effect of the extract via antioxidant effects of the plant as other plants have been reported to exert similar effect because of their antioxidant potential (Okokon et al., 2011). The phytochemical analysis of the plant extract revealed the presence of tannins, alkalioids, flavonoids and cardiac glycosides (Chukwu et al., 2014; Etim et al., 2014). Flavonoids and tannins are some of the most important biochemical compounds with anti-ulcer and gastro protective activities (Wahida *et al.*, 2007).

Different mechanisms have been proposed to explain the gastro protective effect of flavonoids, including an increase in mucosa prostaglandin content and a decrease in histamine decarboxylase. Flavonoids are free radical scavengers that are known to play important role in preventing ulcerative and erosive lesions of gastrointestinal tract (Di carlo *et al*., 1999; Zayachkivsa, 2005). Tannins with its protein precipitating and vasoconstrictory effects could be advantageous in preventing ulcer development. Tannins being astringent may have precipitated microproteins on site of the ulcer thereby forming an impervious protective pellicle over the lining to prevent absorption of toxic substances and resist the attack of proteolytic enzymes.

CONCLUSION

This study has demonstrated that the methanolic root bark extract of *N. imperialis* exhibit antiulcer and antidiarrheal properties, which may be due to its rich phytochemical constituent.

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