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ANTI-DIARRHOEAL PROPERTIES OF AQUEOUS-METHANOL LEAVES EXTRACT OF *NAPOLEONA IMPERIALIS* ON ALBINO WISTAR RATS

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ABSTRACT

Electrolyte balance is key to haemostatic balance. Dehydration is fatal, hence the need to control diarrhoea. This study is aimed at examining the anti-diarrhoeal properties of aqueous methanol fraction of *Napoleona imperialis* leaves on diarrhoeal induced in albino Wistar rats with the view to authenticating the claim of its antidiarrhoeal potency. Standard methods and procedures were followed in this experiment. Twenty albino Wistar rats were randomly grouped into five groups of four rats as follows: Group 1 Induction (castor oil 1ml per rat) only, Groups 2-5 (Induction + 5mg/kg bw loperamide, 150, 300 and 600 mg/kg bw Treatment) respectively. The sample demonstrated the presence of different bio-active compounds. The acute toxicity studies of *Napoliona imperialis* extract indicated no sign of toxicity up to 5000 mg/kg bw. In the defecation test, a significant ($p<0.05$) reduction in the mean number of diarrhoeal faeces was noticed in the group administered with 150 mg/kg body weight and loperamide (5 mg/kg bw). The percentage inhibition of diarrhoeal faeces of standard and treatment at 5, 150, 300 and 600 mg/kg body weight were 75%, 87%, 56% and 30% respectively. In gastrointestinal transit test, the fractions showed a non-dose dependent decrease in the distance travelled by charcoal meal when compared to group 1 (control). Group 4 (300 mg/kg b.w) just like loperamide (group 2) showed a significant ($p<0.05$) decrease in the distance travelled by charcoal meal compared to group 1. The standard control group, the treated groups, 150 mg/kg, 300 mg/kg and 150 mg/kg showed 27.32%, 10.40%, 23.56% and 17.77% inhibitions in the distance travelled by charcoal meal, respectively compared to the control (group 1). In gastro-enteropooling studies, the fraction showed a non-significant ($p > 0.05$) decrease in the weight and volume of the intestinal content in a non-dose dependent manner when compared to the control (group 1). *Napoleona imperialis* has anti-diarrhoeal activity. The study, therefore validates the ethnopharmacological claim of the plant's anti-diarrhoeal potency.

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INTRODUCTION

The most important public health challenge connected to sanitation and water is Diarrhoea. It can be both "water-washed" and "waterborne". Diarrhoea remains the leading cause of morbidity and mortality among children in developing countries. About one billion episodes and approximately 2.5 million deaths occur yearly among children of under the ages

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of five [1]. According to WHO, about 444 thousand deaths occur yearly among children below the age of five worldwide as a result of diarrhoea, mainly in developing countries [2,3,4]. Diarrhoea is among the leading cause of death among children in Nigeria. It has a prevalence rate of 12.9% [5]. This is among the worst in sub-Sahara Africa. Diarrhoea could be defined as the alteration in the normal bowel movement, marked by increase in the frequency of bowel sound and movement, abdominal pain and wet stool [2]. WHO defines diarrhoea as the discharge of three or more watery or loose stools per day [2]. Water is absorbed and secreted into the guts in a finely balanced and dynamic way. Diarrhoea occurs when this balance is lost or due to lack of balance between the secretory and absorptive mechanisms in the intestinal tract followed by hypermotility. This leads to the excessive loss of electrolytes and fluid in stool [6]. We have both infectious and non-infectious causes of diarrhoea. Infectious diarrhoea are mostly caused by virus and bacteria. The common sources of these infections are food and water contaminated with faeces. It could also be gotten directly from an infected person. Non-infectious causes include irritable bowel syndrome, inflammatory bowel disease, lactose intolerance, celiac disease, non-celiac gluten sensitivity and some medications [7].

The absorption of water and electrolytes occur primarily in the small and large intestines. This absorption of water, occur through the process of passive osmosis (commonly through a paracellular route located between the junctions of enterocytes). As a result, the reabsorption of water is primarily facilitated by active absorption of osmotic minerals, especially sodium (Na^+). In a situation where solutes are not absorbed and therefore remain in the intestinal lumen, their high concentration prevent water from being reabsorbed and therefore results in what is termed osmotic diarrhoea.

Napoleona imperialis is a plant found mostly in the South Eastern part of Nigeria. It is evergreen and grows abundantly in the humid and tropical regions of West Africa. It is of the family lecythidaceae. Another popular plant that belongs to this family is the cannon ball tree (*Corrupita guianensis*), which is found in most part of Nigeria. The average height of *Napoleona imperialis* is 14.5m while its girth is about 10m. It has a dark green foliage with a dense rounded crown. The bark has a greyish to pale brown colour which tend to darken on exposure. It produces its fruits around April. The fruits are mostly broad and globular in shape [8].

N. imperialis is reported to be a very rich source of commercial hemolytic saponins and feed ingredient [9]. The extract of *Napoleona imperialis* has also shown some wound-healing and anti-bacterial properties [10,11]. Some reports have also shown that *N. imperialis* has some anti-hypertensive, abortifacient properties and anti-implantation [12]. The leaves also contain some amounts of cyanide, saponins, glycosides, proteins and tannins [13,12]. The roots of *N. imperialis* contain some important phytochemicals like alkaloids, proteins, steroids, flavonoids, tannins, glycoside, saponins, carbohydrate and resin. They also possess some free-radical scavenging properties [12]. Phytochemicals are a group of compounds which belongs to secondary metabolites of plants. They include a wide range of compounds like polyphenols, vitamins, flavonoids, steroidal saponins, and organo-sulphur compounds. They are very useful in pharmaceutical, cosmetics and biotechnology [14].

Diarrhoea induction using castor oil is possible due to the action of ricinoleic acid formed when oil is hydrolized by the intestinal lipase [15]. Ricinoleic acid produces hypersecretory responses due to its alteration of the process by which water and electrolytes are transported in the lumen of the intestine [16]. According to Tunaru et al [17], Castor oil are used to induce uterine contraction and laxation through the help of ricinoliec acid activating prostaglandin receptors (EP_3). While according to Mascolo et al [18], the ricinoleic acid stimulates the production of nitric oxide in both small intestine and large intestine (the effect is felt more in the ileum than inside the colon). The ricinoleic acid which is harmful to epithelial cells of the intestine increases the entry of extracellular calcium into the epithelial cells of the intestine and this could help in the formation of a constitutive form of Nitric oxide synthase thereby contributing to diarrhoea due to the stimulatory effect of nitric oxide.

Nitric oxide is implicated in the mechanism of diarrhoea induction using castor oil. Pre-treatment using NO synthase inhibitors (l-arginine based inhibitors) can prevent castor oil induced diarrhoea and decrease the amount of fluid accumulation and sodium ion secretion in the intestine caused as a result castor oil induction. Nitric oxide is therefore involved as a mediator in intestinal secretion and diarrhoea induction caused by castor oil [19]. Nitric oxide is also implicated in the cholinergic activation of water secretion [20].

Loperamide is an opoid drug used for the treatment of diarrhoea caused by gastroenteritis or inflammatory bowel disease. It acts on the u-opid receptor of the myenteric plexus situated at the large intestine. Loperamide on its own does not interfere with the central nervous system [21]. Loperamide and morphine works in a similar way. It decreases the activity of the myenteric plexus, which then leads to a decrease in the tone of the circular and longitudinal muscles of the wall of the intestine. Consequently, the amount of time taken by substances stay in the intestine is increased in order to allow more water to be absorbed from the of the intestinal content. Loperamide also stimulates absorption and triggers an anti-secretory activity mediated by calmodulin antagonism. This property is lacking in other opoids. .

This work therefore, is aimed at examining the effects of *Napoliona imperialis* on castor oil-induced diarrhoea in Wistar albino rats with the view to authenticating the ethnopharmacological claim that it has anti-diarrhoeal activity.

MATERIALS AND METHODS

Plant Collection and Identification

Napoleona imperialis (fresh leaves) were harvested from Umudikwu Village Nsukka, Enugu State, Nigeria. The

identification of the leaves was done in the Department of Pharmacognosy and Medicinal Chemistry, University of Nigeria Nsukka.

Experimental Animals

Sixty (60) albino rats of either both were used in this experiment. 18 adult albino mice were used for the acute toxicity (LD_{50}) study. The animals were purchased from the Animal House of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were fasted for 18 h before the commencement of the experiment.

Extraction and fractionation of *Napoleona imperialis*

Fresh leaves of *Napoleona imperialis* were shade-dried until crispy with regular turning to prevent decaying. The dried leaves were then ground into coarse form using a grinder. 600 g of the leaves was then soaked in 2.5L of methanol. The mixture was allowed to stand for 48 hours and then filtered using a muslin cloth. Whatman filter paper was used for further filtration in other to remove fine residues. After the filtrations, a rotary evaporator was used to concentrate the filtrate. After the concentration, a crude methanol extract was obtained. The crude methanol extract (20 g) was dissolved in 250 ml of 20% methanol and 250 ml of n-hexane was added, then the mixture vigorously was shaken and allowed to stand in order to ensure complete separation. The n-hexane phase was collected and the process was repeated with 250 ml of n-hexane. Thereafter the n-hexane phase was collected. The aqueous methanol residue was then fractionated again using 250 ml of ethyl acetate. Finally, the different solvent partitions were collected and concentrated to have concentrated aqueous methanol, ethyl acetate and n-hexane fraction. Then the fraction (aqueous methanol fraction) which showed a better result in the pilot study was selected for further studies.

Preparation of Charcoal Meal

0.5 ml of 10 % charcoal was suspended in 5 % gum acacia to get charcoal meal.

Qualitative Phytochemical analysis

The screening for some chemical constituents and their quantities in the plant's leaves was done as described by Harborne [22], and Trease and Evans [23].

Quantitative Phytochemical analysis

Determination of Total Phenolic Content

1 g of the sample was macerated using 20 ml of 80 % ethanol for ten minutes, was centrifuge for 5 minutes. 2 ml of supernatant was then introduced into three different tubes, 3 ml of water was added, 0.5 ml of Folinciocalteus reagent was mixed and allowed to stand for 5 minutes, 2 ml of 20 % Na_2CO_3 carbonate was then added and mixed. The mixture was then allowed to stand for 30 minutes, and the absorbance was read at 760 nm.

Determination of Terpenoid Content

0.5 g of sample was macerated using 10 ml of ethanol for 10 minutes, centrifuged for 5 minutes. 1 ml of supernatant was transferred into three different tubes. 1 ml of 5 % aqueous phosphimolybdic acid solution was added and mixed, 1 ml of conc. H_2SO_4 was gradually mix and allow to stand for 30 minutes. Ethanol (2 ml) was added and the absorbance was read at 700 nm against the blank.

Determination of Tannin Content

1 g of sample was macerated using 20 ml of methanol for 10 minutes, centrifuged for 5 minutes and 2 ml of supernatant was introduced into three different tubes, 3 ml of methanol was added, 0.3 ml of 0.1 M $FeCl_3$ in 0.1 M HCL was added. 0.3 ml of 0.0008 M Potassium ferricyanide was added and mixed. Then the absorbance was read against blank at 720 nm after 5 min but below 30 min.

Determination of Alkaloid Content

1 g of the sample was macerated using 10 ml of ethanol and 10 ml of 20 % H_2SO_4 for 10 minutes. The mixture was spun in a centrifuge for 5 minutes, 0.5 ml of the supernatant was then transferred into three different tubes and then 2.5 ml of 60 % H_2SO_4 and 2.5 ml of 0.5 % formaldehyde in 60 % H_2SO_4 were added, mixed thoroughly and allowed to stand for 3 hours, then absorbance read at 565 nm against the blank.

Determination of Flavonoid Content

1 g of the sample was macerated with 20 ml of ethyl acetate for 10 minutes and spun using a centrifuge for 5 minutes. 5 ml of the supernatant was then introduced into three test tubes and shaken vigorously for about 2 to 5 minutes. It was spun for 5 minutes, and the upper layer was discarded while the lower layer was collected. The absorbance of the lower layer was then read at 470 nm.

Determination of Glycoside Content

A 1 g of the sample was macerated using 20 ml of water, 2.5 ml of 15 % lead (II) acetate for 10 minutes. It was filtered, 10 ml of $CHCl_3$ was added to the filtrate and shaken for 5 minutes, centrifuged for 5 minutes. Then 2.5 ml of the lower layer was transferred into three tubes. The content of the tube was dried by evaporation and allowed to cool. The residue was then dissolved using 3 ml acetic acid. Then 0.1 ml of 5 % $FeCl_3$ was added and mixed thoroughly. 0.25 ml of concentrated H_2SO4 was then added and then thoroughly mixed. The mixture was kept in the dark for 2 hr and the absorbance read at 530 nm against the blank.

Determination of Steroid Content

The sample (1g) was macerated using 20 ml petroleum ether for 10 min and allowed to stand for 1 hr while shaking intermittent after every 10 minutes, spun in a centrifuge for 5 minutes. 2 ml of the supernatant was then put into three tubes and allowed to evaporate to dryness, then 2 ml of alcoholic KOH was added and allowed to boil for 30 minutes. After cooling, 3 ml of petroleum ether was then added and shaken for 2 min, then spun for 5 min. 2 ml of the upper layer was then transferred into another tube and then made to evaporate to dryness. After cooling, 2 ml of ethanol was added to dissolve the residue, steroid color reagent (2ml) was added and vigorously shaken and allowed for a period of 30 min to stand. The absorbance was then read at 550 nm against the blank.

Acute Toxicity Test

Lorke's method [24] was utilized for the toxicity study of the methanol extract of *Napoleona imperialis*. 18 albino mice were used for the study. Two stages were involved in the test. Stage one had three groups of three mice each. They were given 10, 100 and 1000 mg per kilogram body weight of the extract respectively. After 24h, the 2nd stage followed. In stage two, 1600, 2900 and 5000 mg per kilogram body weight of the extract were given to the animals respectively. The extracts were administered orally.

Anti Diarrhoeal Studies

Castor oil induced diarrhoea

Effects of aqueous methanol fraction of *Napoleona imperialis* on castor oil-induced diarrhoea was evaluated in the albino rats utilizing the method of Awouters et al [25]. 20 adult albino rats, which previously had been fed standard Pfizer diet, and allowed access to water, were utilized. The Albino rats were placed on fasting but allowed access to water. Then after 18hrs of the fasting, they were divided into five groups of four rats each and then treated as shown below:

Group 1; administered with 3mg/kg of distilled water (control).
Group 2; administered with 5 mg/kg of loperamide, the standard drug.
Group 3; administered with 150 mg/kg b. w aqueous methanol fraction of *Napoleona imperialis*.
Group 4; administered with 300 mg/kg b. w aqueous methanol fraction of *Napoleona imperialis*.
Group 5; administered with 600 mg/kg b. w aqueous methanol fraction of *Napoleona imperialis*.

Exactly 1 h after administration, groups 1-5 rats were given 1 ml of castor oil orally. The rats at this point were separated into their individual metabolic cages. This was followed by keen observation of the rats for consistency of faecal discharge and frequency. The faeces were then collected on a white paper placed beneath the metabolic cages. The number of wet and dry faeces for each rat was taken every 1 hour for 4 hours and the white papers were evaluated and changed periodically. The number of wet and dry stools showed the degree of wetness of stool discharged and the frequency of the discharge. Finally, the percentage of diarrhoeal inhibition was calculated with the formular shown below.

$$\% \text{ of inhibition} = \frac{Mean\ number\ of\ WFC - Mean\ number\ of\ WTF}{Mean\ number\ of\ WFC} \times 100$$

WFC stands for wet faeces in control; While WFT stands for wet faeces in test group

Gastro-intestinal motility

Effects of aqueous methanol fraction of *Napoleona imperialis* on gastro intestinal motility was studied in the rats with the method of Mascolo et al [18]. 20 adult albino rats which were fed previously with standard Pfizer diet and given access to water, were utilized. The Rats were subjected to fasting with unrestricted access to water. After 18hrs of fasting, the animals were separated into five groups of four rats each. Then all groups were administered 1ml castor oil to produce diarrhoea.

Exactly 1 h after the administration, the rats in groups 1-5 were given 1 ml of charcoal meal (a suspension of 10 % in 5 % gum acacia) orally. 1 h after, every of the rats was sacrificed by abdominal incision. Then the small intestine was separated out carefully starting from the mesentrum so as to avoid stretching. The length of the intestine from the pyloric sphincter to the ileo-caecal junction was measured. The distance travelled by the charcoal meal was also measured for each of the rats. The gastro-intestinal transit which is calculated by checking the percentage distance travelled by the charcoal meal relative to the length of the intestine was calculated for each rat. The peristaltic index/percentage intestinal transit and percentage inhibition were also calculated with the formular shown below;

$$\text{Percentage intestinal transit/peristaltic index} = \frac{Distance\ travelled\ by\ charcoal\ meal}{Lenght\ of\ small\ intestine} \times 100$$

$$\% \text{ inhibition} = \frac{Dc - Dt}{Dc} \times 100$$

Dc stands for the Mean distance travelled by the control; While Dt represents the Mean distance travelled by the test group.

Castor Oil-Induced Enteropooling Studies

The determination of the effects of aqueous methanol fraction of *Napoleona imperialis* on castor oil-induced enteropooling was done with the method of Robert et al [26]. 20 adult rats which were previously fed with standard diet and given unrestricted access to water were utilized. The rats were placed under fasting for 18hr while allowing them unrestricted freedom drink to water. They were divided into five groups of 4 rats each

1hr after the administration above, 1 ml castor oil was given to rats of groups 1-5 each orally. 1hr later, the animals were sacrificed. The small intestine of each rat was located, tied at the pyloric sphincter and the caecal junction and cut out. After the sacrifice, the contents of the intestines were milked out and weighed. The volume was also measured with a graduated tube.

Statistical analysis

Statistical analysis of the raw data was done with SPSS version 20. Test of significance was carried out at $P < 0.05$. Statistical differences were evaluated using a one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test to detect the significant difference among the values of the different groups.

RESULTS

Median Lethal Dose of *Napolina imperialis* extract

The toxicity studies of *Napoleona imperialis* shown in Table 1 reveals that there was no observed sign of toxicity or death in the 1st phase. Similarly, in the 2nd phase where the animals were treated with doses up to 5000 mg per kilogram, there was no sign of death or toxicity.

Qualitative phytochemical content of *Napoleona imperialis* leaves

Qualitative phytochemical analysis of *Napoleona imperialis* leaves revealed that phenolics, alkaloids, flavonoids were of high amount while glycosides were of moderate quantities. Steroids, tannins and terpenoids are of little quantities.

Quantitative Phytochemical Compositions of *Napoleona imperialis* leaves

The quantitative phytochemical studies of *Napoleona imperialis* leaves in table 3 shows a decreasing order of abundance of the following constituents: Total phenolics > flavonoids > alkaloids > glycosides > terpenoids > tannins > steroids.

Inhibition of diarrhoea by aqueous methanol fraction of *Napoleona imperialis*

The levels of inhibition of diarrhoeal stooling caused by *Napoleona imperialis* compared to the untreated control are shown in Table 3. A significant reduction ($p < 0.05$) in the mean number of diarrhoeal faeces was witnessed in group treated with 150 mg/kg b. w. A significant reduction ($p < 0.05$) in the mean number of wet faeces when treated with loperamide was also witnessed. However, there was a non-significant reduction ($p > 0.05$) in the number of wet faeces when 300 mg/kg 600 mg/kg b. w of the fraction was administered. There was an inverse effect of the extract on castor oil-induced diarrhoea. The percentage inhibition of diarrhoeal faeces at 150, 300 and 600mg/kg body weight were 86.66 %, 53.33 % and 26.67 % respectively. Loperamide, the standard drug, showed a 73.33 % inhibition of diarrhoeal faeces. The table also shows the mean values of total defecation produced in group 1 to 5. Here the 20 % methanol fraction of *Napoleona imperialis* showed a non-significant decrease on the total number of faeces over a period of 4 hours compared to the normal control.

Effects of aqueous methanol fraction on intestinal transit of charcoal meal

Table 4 shows the effects of the fraction on gastro-intestinal motility in albino rats, using charcoal meal as the marker. The methanol fraction showed a non-dose dependent decrease in the distance travelled by the charcoal meal when compared to the group 1 (negative control). The fraction of *Napoleona imperialis* significantly ($P < 0.05$) decreased the propulsion of charcoal meal in rats' gastrointestinal tracts by 300 and 600 mg/kg bw compared to the negative control. A similar significant reduction ($P < 0.05$) in the gastrointestinal transit of charcoal meal in rats was achieved with the reference drug, loperamide. There was no significant ($p>0.05$) difference between the reductions in the distance travelled by charcoal meal in groups 3, 4, 5 when compared with the reference drug, loperamide. Group 2, 3, 4, 5 showed 27.32%, 10.40 %, 23.56% and 17.77% inhibitions of the distance travelled by charcoal meal respectively compared to group 1.

Effects of aqueous methanol fraction on enteric content.

Tables 6 show the effect of the extract on the volume and weight of the intestinal content of Wistar albino rats. The standard and fraction showed a non-significant ($p > 0.05$) decrease in the volume and weight of intestinal content in a non-dose dependent manner when compared to the control group.

Table 1: The median lethal dose of the methanol extract of *Napoleona imperialis*.

	Doses (mg/kg b.w)	Motality
Phase 1		
Grp 1	10.00	0/3
Grp 2	100.00	0/3
Grp 3	1000.00	0/3
Phase 2		
Grp 1	1600.00	0/3
Grp 2	2900.00	0/3
Grp 3	5000.00	0/3

Table 2: Qualitative phytochemical composition of *Napoleona imperialis* leaves

Phytochemicals	
Flavonoids	**+++**
Alkaloids	**+++**
Glycosides	**++**

Terpenoids	**+**
Tannins	**+**
Steroids	**+**

Key: + = Slightly present; ++ = present in a moderate amount; +++ = present in High amount

Table 3: Quantitative Phytochemical Composition of *Napoleona imperialis* leaves

Phytochemicals	**(mg/100g)**
Steroids	**0.21±0.04**
Terpenoids	**16.16±0.20**
Tannins	**9.32±1.70**
Glycosides	**64.69±2.72**
Total phenolics	**4672.04±44.00**
Flavonoids	**371.33±1.04**
Alkaloids	**386.95±51.00**

Results are expressed in Mean ± SD; n = 3

Table 4: Effect aqueous methanol fraction on castor oil-induced diarrhoea

Group	**Number of wet stools**				**Wet & dry Faeces**	**Diarrhoeal faeces**	**Inhibition of wet stool (%)**
	1st h	**2nd h**	**3rd h**	**4th h**			
Untreated control	2.00	8.00	3.00	2.00	6.50±3.11^{a}	3.75±2.87^{b}	-
Loperamide (5 mg/kg)	2.00	2.00	0.00	0.00	1.25±2.65^{b}	1.00±1.15^{a}	73.33
AMFNI (150 mg/kg)	0.00	0.00	2.00	0.00	2.25±2.63^{a}	0.50±1.00^{a}	86.67
AMFNI (300 mg/kg)	0.00	3.00	1.00	3.00	5.25±2.22^{a}	1.75±1.50^{b}	53.33
AMFNI (600 mg/kg)	2.00	3.00	4.00	2.00	3.00±3.11^{a}	2.75±0.96^{b}	26.67

Result expressed as Mean±SD (n=4); Mean values having different lower-case letters as superscripts within the column are significant ($p < 0.05$).

Table 5: Effects of Aqueous methanol fraction on intestinal transit of charcoal meal.

Group	**Total length of intestine (cm)**	**Distance travelled by charcoal meal**	**Percentage intestinal transit**	**Inhibition of charcoal transit (%)**
Negative control	83.34±3.94	75.25±7.98	90.14±6.73^{a}	-
Loperamide(5mg/kg)	84.5±1.73	55.25±12.52	65.51±15.40^{b}	27.32
AMFNI (150 mg/kg)	85.50±5.00	68.63±10.55	80.76±14.86^{a,b}	10.40
AMFNI (300 mg/kg)	82.30±4.7	56.50±2.54	68.90±5.74^{b}	23.56
AMFNI (600 mg/kg)	85.50±1.2	63.38±10.67	74.12±3.06^{b}	17.77

Result expressed as Mean±SD (n=4). Mean values having different lowercase letters as superscripts across the column are considered significant ($p < 0.05$).

Table 6: Effects of aqueous methanol fraction on the volume and weight of intestinal content.

Groups	Volume of Intestinal Fluid (ml)	Weight of intestinal fluid (ml)
Group 1 induction only	1.00±0.63[a]	1.40±0.85[a]
Group 2 Induction + Std 5 mg/kg b. w	0.73±0.32[a]	0.85±0.30[a]
Group 3 Induction + Extract 150 mg/kg b. w	0.88±0.41[a]	0.90±0.38[a]
Group 4 Induction + Extract 300 mg/kg b. w	0.80±0.56[a]	0.70±0.48[a]
Group 5 Induction + Extract 600 mg/kg b. w	0.93±0.15[a]	0.90±0.12[a]

Result expressed as Mean±SD (n=4). Mean values having different lower-case letters as superscripts across the column are considered significant ($p < 0.05$).

DISCUSSION

Electrolyte balance is key to haemostatic balance. Dehydration is fatal hence the need to control frequent stooling that is common in diarrhoeal episodes. This present work was aimed at studying the anti-diarrhoeal activity of the aqueous methanol fraction of *Napoleona imperialis* leave extract using different experimental models of diarrhoea in rats.

The acute toxicity test of the extracts of *Napoleona imperialis* showed no sign of toxicity up to 5000mg/kg b. w which suggested that the plant is relatively safe for human consumption.

The preliminary phytochemical screening revealed a high quantity of phenolics, alkaloids, flavonoids; moderate amounts of glycosides, and little quantity of other secondary plant metabolites like steroids, tannins and terpenoids in *Napoleona imperialis*. Some of these plant metabolites are known to have antidiarrhoeal property while some work synergistically with others to bring about an antidiarrhoeal effect [27]. Tannins are reported to reduce diarrhoea by reducing intestinal secretion by denaturing the secretory proteins and inhibiting intestinal transit through the alteration of intracellular calcium ion level [28]. Certain flavonoids like quercetin-3-O-β-D-glucopyranoside are reported to also inhibit intestinal transit, electrolytes and water secretion (29). They inhibit secretions caused by prostaglandin E2 and contractions caused by spasmogen [30,31]. They also have antioxidant properties which may work in inhibiting the enzymatic activities of some enzymes including those that help in the synthesis of prostaglandins and those implicated in metabolism of arachidonic acid [32]. Steroids are also known to encourage hydroelectrolytes absorptions across the lumen of intestine [33].

Aqueous methanol fraction of *Napoleona imperialis* successfully inhibited the defecation of diarrhoeal faeces in an inverse manner with the lowest dose having the highest effect. The lower the dose, the more effective the fraction in inhibiting the defecation of diarrhoeal/wet feaces. This contradicts the work of Kola-mustapha st al [34] on the anti-diarrhoeal activity of the leaf extract of *Paequetina nigrescens* where the *Paequetina nigrescens* leaf extract showed an increasing effect with increasing dose on the inhibition of wet faeces in a diarrhoea induced mice. The extract, also successfully postponed the onset of diarrhoea in an inverse order with lowest dose of the fraction showing the highest effect in the postponement of diarrhoea onset. The inverse effect of the extract could be due to the inhibitory activities of the component(s) of the extract which led to the reduction of the activity of the extract as the doses increases. According to Caesar and Cech [35] studies have shown that the total biological activity of plant extracts can result from combination of compounds with additive, synergistic or antagonistic activity.

The *Napoleona imperialis* fraction successfully inhibited intestinal transit of charcoal meal and increased the transit time in a non-dose dependent manner. This is in agreement with the work of Igboeli at al [36] on the antidiarrhoeal activity of methanol leaf extract of *Lophira Lanceolata Tiegh* (Ochnaeceae). This behaviour could be as a result of receptor saturation or due to external influence. Pre-treatment with *Napoleona imperialis* fraction reduced the propulsion of charcoal meal through tract of the intestine. This delay in intestinal motility increases the time of stay of the contents in the intestine and this may promote intestinal water and electrolyte absorption. So, the antidiarrhoeal activity of the plant extract may be due to or partly ascribed to its anti-motility property [37]. They also have anti-spasmolytic effect. The contraction of the gastrointestinal muscles leads to the increase in the propulsive movement of the content of the intestine thereby decreasing the transit time. This does not allow enough time for nutrients, fluid and electrolytes to be reabsorbed thereby leading to watery faeces. Contraction is induced when acetylcholine binds to the parasympathetic receptors. The fraction may also have some phytochemicals like alkaloids which possess a muscle relaxant and anti-cholinergic activities. It could be due to the presence of phenolics like tannins and flavonoids which can inhibit acetylcholine and histamine induced contractile responses.

According to Parker et al [38], loperamide exerts its anti-peristaltic or anti-motility activities by inhibiting acetylcholine release through interacting with opiate receptor sites in the myenteric plexus and also by inhibiting the synthesis of prostaglandins. The inhibition of the release of prostaglandin

may be as a result of the inhibition of prostaglandin production in the intestine because loperamide prevented the biosynthesis of prostaglandin from arachidonic acid. They also discovered that loperamide hinders the propulsive movement of the intestinal content by reducing the release of acetylcholine and prostaglandin, at least during circumferential distension of the walls of the intestine *in vitro*.

The enteropooling test showed a minimal decrease in the volume of enteric contents when different doses of *Napoleona imperialis* fraction were administered. It shows that at doses 150 mg/kg, 300 mg/kg, 600 mg/kg body weights, the mean volume of enteric contents includes 0.73±0.32, 0.88±0.41 and 0.93±0.15 respectively. The fraction showed the maximum effect at 300mg/kg while the least effect was noticed at the highest dose 600 mg/kg aqueous methanol fraction of *Napoleona imperialis*. This activity of the extract could also be as a result of the maximum occupation of the receptor binding site by the fraction of the extract or the inhibitory effect of the phytochemical components of the fraction on the main active component of the fraction. A similar trend observed in the volume of the content of the intestine was also observed in the weight of the intestinal content at the different doses.

The anti-diarrhoeal activity of *Napoleona imperialis* could be as a result of the anti-motility and anti-secretory component of the fraction.

CONCLUSION

From the findings, aqueous methanol extract of *Napoleona imperialis* exhibited antidiarrhoeal properties by inhibiting gastro-intestinal motility, enteropooling, wetness and frequency of defecation. The study, therefore affirms the traditional use of *Napoleona imperialis* in the treatment of diarrhoea.

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AUTHORS' CONTRIBUTION

Eze Ifeanyi Harford designed and carried out the work. Njoku Ugochi Olivia supervised the work. Oguguofor Martins provided technical support. Abonyi Obiora Emmanuel Technical support and manuscript drafting.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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