Assessment of the Potential Mechanisms of Anti-diarrhoeal Activities of ethanol extract of Polyantha logofolia Leave in Wistar Rat.

ABSTRACT

Background: Diarrhea illnesses are one of the main reasons for morbidity and mortality in developing nations and are accountable for the death of hundreds of thousands of people every year. *Polyalthia longifolia*, the false ashoka, also known as *Monoon longifolium* is a tree species from Asia. It is commonly found in southern india, Sri Lanka and Nigeria. Traditionally, people use *Polyalthia longifolia* for the treatment of various ailments, including diarrhea.

Methods: The plant was extracted with ethanol using cold maceration. Preliminary qualitative and quantitative phytochemical screening of ethanol extract of *Polyalthia longifolia* leaf was carried out according to the method of Harbone (1998); Trease and Evans (1973). Median Lethal Dose (LD_{50}) was carried out according to the method of Lorke (1983). Castor oil-induced diarrhea was carried out by method of Awouter *et al.,* (1973). Gastrointestinal transit assay was carried out method of Mascolo *et al* (1996). Electrolyte levels assay was carried out by method of Tietz (1994). Kidney function markers assay was carried out according to the method of Bartels and Bohmer (1972).

RESULTS: The results of phytochemical screening showed that alkaloids and tannins occurred in high concentration, flavonoids and steroids occurred in moderate concentration, whereas terpenoids, glycosides and phenolic acid occurred in lowest concentration. The acute toxicity study revealed that the plant extract was not toxic even at the dose of 5000 mg/kg. Groups treated with 200,400, and 600 mg/kg b.w of extract showed significant ($P < 0.05$) inhibition in the frequency of defecation of wet feces and total fecal output compared with positive control. Similarly, Groups treated with 200, 400, and 600 mg/kg body weight of extract demonstrated significant ($P < 0.05$) antimotility activity when compared with the positive control. However, groups treated with 200,400 and 600mg/kg body weight showed a significant ($P < 0.05$) increase in chlorine and sodium with corresponding decrease in bicarbonate and potassium level when compared with the positive control. The results from kidney function markers showed that groups treated with 200, 400 and 600 mg/kg b.w in a dose dependent manner showed a significant ($p < 0.05$) reduction in creatine and urea when compared with positive control.

Conclusion: The ethanol leaf extract of *Polyalthia longifolia* has considerable antidiarrheal activity on castor oil-induced diarrhea and gastrointestinal motility models, confirming the reason for its wide use in traditional treatment of diarrheal conditions.

Key words: Diarrhoea, electrolytes, gastrointestinal motility, Polyanltha logifolia, ethanol extract

Introduction

Numerous thousands of people die each year from diarrheal infections, which are a major cause of morbidity and mortality in underdeveloped countries.[1] According to a 2015 estimate, diarrhoea is one of the leading causes of death for children, accounting for 9% of all paediatric fatalities globally.[2] This report states that the regions with the highest rate of diarrhea-related child deaths

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were sub-Saharan Africa and southern Asia.3. In Ethiopia, one of the top 15 nations where diarrheal sickness accounts for approximately three-fourths of child fatalities, the illness is

a significant public health concern.[3] .Overall, despite numerous governments' and international organisations' best efforts to lower it, the prevalence of diarrheal illness remains high.[4]. Despite significant advancements in medical technology, 80% of people in developing nations still get their daily medical treatment from traditional healers and medicinal plants.[5] .Similarly , Nigeria has long used plants as a source of medications to treat a variety of ailments that affect both people and their animals.[6]. Because of its natural origin and fewer side effects, the use of herbal therapy is becoming more and more common in both developed and developing countries. Additionally, natural goods have proven successful in the drug-development process; currently, over 50% of the most popular prescription medications are derived from herbal medicines.[7]. As a result, relying on conventional medical practice, the World Health Organisation (WHO) promoted research for the prevention and treatment of diarrheal disorders.[8] Through its Diarrhoea Control Programme, the World Health Organisation has used traditional medicine to address the effects of diarrhoea.[9]. The side effects of opioid-like antimotility medications are limiting their use and encouraging scientists to look for novel antidiarrheal substances with a variety of chemical constitutents. More scientists are focusing on traditional medicine in an effort to expand the range of medications available for treating diarrheal illnesses.[10]

Polyalthia longifolia, the false ashoka, also commonly known by its synonym *Monoon longifolium,* is a tree species from Asia and its family is Annonaceae. It is commonly found in southern India and Sri Lanka.[11]. It has been reported that the bark and leaves of this plant show effective antimicrobial activity, cytotoxic function, antiulcer activity, hypoglycemic activity, and hypotensive effect. [12]. This plant has been mentioned by traditional healers in treatment of ulcer, inflammation, analgesic, diarrhea and therefore have been claims of considerable success with the use of this plants. Consequently, this would have a great potential for the finding of an antidiarrhea agent. Therefore, the present study was undertaken to determine the anti-diarrhea activity of ethanol extract of *Polyalthia longifolia* leaf in rats.

MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant materials

The leaves of *Polyalthia longifolia* leaves were used for this study. The leaves were collected from Oba in Nsukka Local Government Area in Enugu state, and were identified by Mr. Alfred

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Ozioko of Bioresource Development and Conservation Programme (BDCP) research centre, Nsukka, Enugu State**.**

2.1.2 Animals

Thirty (30) adult male Albino rats of weight (170-220 g) were used for the antidiarrheal studies and eighteen adult male Albino mice of weight (16-26 g) were used for the median lethal dose (LD_{50}) study. All the animals were obtained from the animal house of the Department of Veterinary Medicine, University of Nigeria Nsukka. The rats were fed with water and standard Growers mash rat pellets (Grand Cereals LTD, Enugu).

2.1.3 Instruments/Equipment:

The equipment used were obtained from the Department of Biochemistry, University of Nigeria Nsukka, Bishop Shanahan Hospital, Nsukka and other scientific shops in Nsukka. They include the following: Centrifuge (PAC, Pacific), Beakers (Pyrex), Conical flask (Pyrex),Filter paper (Whatman), Micro pipette (Perfect), Refrigerator (Haier thermocool), Spatula (Pyrex), Colorimeter (Techmel and Techmel), Spectrophotometer (E312 Model, Jenway, UK), Syringe (Life Scan), Grinding mechine, Incubator, Thermometer (Zeal), Water bath(Gallenkamp, London), Weighing balance (Metler HAS), Hand gloves, Metre rule, Razor,

2.1.4 Chemicals/reagents

The chemicals and reagents used for this research work were of analytical grade. The chemicals and reagents used in this study include: Absolute ethanol (Sigma, London), Twin 80, Sulphuric acid (BDH, England), Castor oil(Goya, Nigeria), Distilled water(Department of Industrial Chemistry, UNN), Randox Kits (USA), Teco (TC) Kits (USA), Fehlings solution A and B (BDH, England), Aluminum chloride (BDH, England), Ethyl acetate (BDH, England), Picric acid (Lab Tech chemicals, London), Sodium chloride, Iodine crystal (Merck), Potassium iodide (East angelis), Glacial acetic acid (Sigma, London), Mercuric chloride (Sigma, London),

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2.2. Methods

2.2.1 Preparation of plant material

The fresh leaves of *Polyalthia longifolia* were air dried and pulverized to coarse powder. Pulverized leave (1000 g) were macerated in 5 liters of ethanol for 72hours. The suspension was filtered with china white, followed by Whatman No. 1 filter paper. The filtrate was concentrated at 64^{oC} using a water bath. Then, the extract was evaporated into slurry form. The Percentage yield of the extract was calculated thus:

$$\text{Percentage yield} = \frac{\text{weight of the crude extract}}{\text{Weight of the pulverized extract}} \times 100$$

2.2.2 Determination of median lethal dose

The acute toxicity test of ethanol leaf extract of *Polyalthia longifolia* was conducted in accordance with the method of [13]. The study was conducted in two phases using a total of 18 mice. In the first phase, nine mice were divided into 3 groups of 3 mice each. Groups 1, 2, 3 animals were treated with 10, 100 and 1000 mg/kg body weight (b.w) of the extract respectively. Clinical signs of toxic effect and mortality were observed within 24 hrs.

In the second phase, 9 mice were divided into 3 groups of 3 mice each. Three groups of three (3) mice each were treated with 1600, 2900, and 5000 mg/kg b.w of the extract respectively. The extract was dissolved in normal saline and the route of administrate was oral (p.o).

2.2.3 Experimental design:

Thirty (30) adult male albino rats, which had previously been fed standard Pfizer diet and allowed free access to water, were used. Rats were fasted for 18 hours with free access to water were divided into six (6) groups of five (5) rats each and treated using suitable stomach tubes as follows.

Group 1: Received 0.2 ml of normal saline (control)

Group 2: Received 1 ml of castor oil (positive control)

Group 3: Administered 2mg/kg of a standard drug, loperamide before castor oil administration

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Group 4: Rats treated with 200 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia* before castor oil administration

Group 5: Rats treated with 400 mg/kg body weight of ethanol leaf extract *Polyalthia longifolia* before castor oil administration

Group 6: Rats treated with 600 mg/kg body weight of ethanol leaf extract *Polyalthia longifolia* before castor oil administration

2.3. Antidiarrheal studies:

2.3.1. Determination of castor oil-induced diarrhea : The effect of ethanol leaf extract *Polyalthia longifolia* on castor oil-included diarrhea was evaluated in rat using the method of [14]. Thirty (30) adult male albino rats, which had previously been fed standard Pfizer diet and allowed free access to water, were used. Rats were fasted for 18 hours with free access to water, divided into six (6) groups of five (5) rats each and treated using suitable mouth gauge needle as follows.

Group 1: Received 0.2 ml of normal saline (control)

Group 2: Received 1 ml of castor oil (positive control)

Group 3: Administered 2 mg/kg of a standard drug, loperamide.

Group 4: Rats treated with 200 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.

Group 5: Rats treated with 400 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.

Group 6: Rats treated with 600 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.

One hour after the above treatment each rat in groups 3-6 received 1ml of castor oil orally and the rats were at this stage separated into their respective individual metabolic cages. The rats were then observed for consistency of fecal discharge and the frequency of defecation. The number of both wet and dry droppings for each rat was counted every 1 hour for a period of 5 hours and the white paper were changed periodically for each evaluation. The number of wet and dry feces counted indicated the degree of wetness and the frequency of defaecation.

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2.3.2 Determination of gastro intestinal motility

The effect of ethanol leaf extract of *Polyalthia longifolia* on gastro intestinal motility was determined in rats using the method of [15]. Thirty (30) male adult albino rats, which had previously been fed standard Pfizer diet and allowed free access to water, were used. Rats were fasted for 18 hours with free access to water, divided into six (6) groups of five (5) rats each and treated using gauge needle as follows:

Group 1: Received 0.2ml of normal saline (control)

Groups 2: Received 1ml of castor oil (positive control)

Group 3: Administered 2 mg/kg of a standard drug, loperamide.

Group 4: Rats treated with 200mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.

Groups 5: Rats treated with 400mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.

Group 6: Rats treated with 600mg/kg body weight of ethanol extract of *Polyalthia longifolia* leaves.

One hour after the above treatments, each rat in groups 2-6 received 0.2 ml of charcoal meal (10% activated charcoal suspended in 5 g of gum acacia). After one hour, each rat was anaesthetized with chloroform, sacrificed by abdominal incision and its small intestine carefully separated from the mesentery in order to avoid being stretched. The length of the intestine from the pyloric sphincter (pylorus) to the ileo-caecal junction (caecum) and the distance travelled by the charcoal meal were measured for each rat. The gastro intestinal transit was calculated for each rat as the percentage distance travelled by the charcoal meal relative to the length of the intestine.

2.4 Electrolytes test

The effects of ethanol leaf extract of *Polyalthia longifolia* on the electrolyte concentration of the intestinal fluid were determined in rats using the method of [16]. Thirty adult male albino rats, which had previously been fed standard Pfizer diet and allowed free accccess to water, were used. Rats were divided into six (6) groups of five (5) rats each and fasted for 18 hours. Each rat

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received 1 ml of castor oil orally using suitable intubation tubes and after one hour, rats in different groups were treated as follows:

Group 1: Received 0.2 ml of normal saline (control)

Groups 2: Received 1 ml of castor oil (positive control)

Group 3: Administered 2 mg/kg of a standard drug, loperamide.

Group 4: Rats treated with 200 mg/kg body weight of ethanol leaf extract of *Polyaltiha longifolia*.

Groups 5: Rats treated with 400 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.

Group 6: Rats treated with 600 mg/kg body weight of ethanol extract of *Polyalthia longifolia* leaves.

One hour after the above treatment each rat in groups 3-6 received 1ml of castor oil orally. Two hours after the treatments, each rat was anaesthetized with chloroform. The rats were sacrificed and their small intestine located and tied at the pyloric sphincter and ileo-caeccal junction. The small intestine of each rat was cut out and the content milked out into a test tube. The effluents from intestinal loops (serosal solution) of the rats were centrifuged at three thousand angular acceleration (3000 G), for 30 minutes. The supernatants were obtained and analysed procedurally for concentrations of Na^+ K^+ and HCO_3^- and Cl^-

2.4.1 Determination of sodium Ion (Na) concentration (Teco diagnostic kit)

The determination of sodium ion concentration was carried out using the method of [16] in which sodium is precipitated as a triple salt, sodium magnesium uracyl acetate, with the excess uranium being reacted with ferrocyanide, producing a colour chromophore whose absorbance varies inversely as the concentration of the sodium in the test specimen. Different test tubes were labeled as standard (S), control (C), test (T) and blank (B). The filtrate reagent (1.0 ml) was pipetted into all the test tubes. An aliquot (50 µl) of the extract was added to all the test tubes while distilled water was added to the blank. All the test tubes were shaken vigorously and mixed continuously for 3 minutes. They were centrifuged at high speed of one thousand five hundred angular acceleration (1500 G) for 10 minutes. A volume of 1.0ml of acid reagent was pipetted into all the

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test tubes at the colour development stage. The supernatant (50 µl) was added to respective tubes and mixed thoroughly. This was followed by the addition of (50 µl) of the colour reagent to all the test tubes and mixed thoroughly. The spectrophotometer was blanked with distilled water after which the absorbance of all the test tubes were read at a wavelength of 550 nm. The concentration of sodium ion in milliequivallent per litre (mEq/L) was calculated using the formular:

$$\text{Concentration of } Na^{+} = \frac{\text{Abs of blank-Abs of sample}}{\text{Abs of blank-Abs of standard}} \times \text{concentration of standard.}$$

2.4.2 Determination of potassium ion (K^{+}) Concentration (Teco diagnostic kit)

The determination of potassium concentration was carried the using the method of [16]. The amount of potassium is determined using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension, the turbidity of which is proportional to the concentration of potassium in the test specimen. The test tubes were appropriately labeled as standard, control and test. The blank was also provided. A volume of 1.0 ml of potassium reagent was pipetted into all the test tubes. Also, 0.01 ml (10 µl) of sample was added to the respective test tubes and mixed thoroughly. These mixtures were allowed to stand at room temperature for 3 mins after which the wavelength of the spectrophotometer was set at 500 nm and blanked. The absorbance of the each solution in the different test tubes was read at this wavelength. The concentration of potassium ion in milliequivallent per litre (mEq/L) was calculated using the formular below:

$$\text{Concentration of } K^{+} = \frac{\text{Abs of blank-Abs of sample}}{\text{Abs of blank-Abs of standard}} \times \text{concentration of standard.}$$

2.4.3 Determination of bicarbonate ion (HCO_3^{-}) concentration (Teco diagonistic Kit).

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Carbon dioxide in serum or plasma exists primarily as dissolved CO_2 and bicarbonate anion (HCO_3). The CO_2 reagent measures CO_2 content enzymatically and the procedure is a modification of the method of [17].

Principle: Phosphoenol pyruvate + HCO_3^- $\xrightarrow{PEPC}$ Oxalate+H_2PO_4

Oxalate $^{++}$NADH $\xrightarrow{MDH}$ Malate + NAD

Phosphoenol pyruvate carboxylase (PEPC) catalyses the reaction between phosphoenol pyruvate and carbon dioxide (bicarbonate) to form oxaloacetate and phosphate ion. Oxalacetate is reduced to malate with simultaneous oxidation of an equimolar amount of reduced nicotinamide adenine dinucleotide (NADH) to NAD. The reaction is catalyzed by malate dehydrogenase (MDH). This results in a decrease in absorbance at 340 nm that is directly proportion to CO_2 concentration in the sample.

Procedure: CO_2 reagent was prepared according to reagent preparation. Test tubes were labeled blank, standard, control, patients, etc. A known quality 1ml of carbon dioxide regent was pipetted into each tube and all the tubes were incubated for 3 minutes at $37^{oC.}$ The spectrophotometer was set at a wavelength of 340nm to a temperature of $37^{oC..}$ 0.005 ml of water, standard and sample were pipette into the cuvette labeled blank, standard, and patients respectively. The contents were then mixed gently by inversion and incubated for 5 minutes. The absorbance of all cuvettes were read and recorded at 340 nm.

Calculation:

$$Co_2 \text{ content of sample (mmol)} = \frac{\text{Abs of blank-Abs of sample}}{\text{Abs of blank-Abs of standard}} \times \text{conc. of standard.}$$

2.4.4 Determination of chloride ion

The concentration of Chloride ion was determined using the method of [18] as outlined in Teco kit.

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Principle

Chloride ions form a soluble, non-ionized compound. When reacted with mercuric ions they would displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions reacted with ferric ions to form a coloured complex that absorb light at 480 nm.
The intensity of the colour produced was directly proportional to the chloride ion concentration.

$Hg(SCN)_2 + 2Cl^-$ $HgCl_2 + 2SCN^-$ ⟶

⟶ $3SCN^- + Fe^{3+}$ $4Fe(SCN)_3$ red complex

Procedure

Two test tubes were labeled blank and calibrator

One and half milliliter (1.5 ml) of chloride reagent (Mercuric Nitrate 0.058 mM, Mercuric Thiocyanate 1.75 mM, Mercuric Chloride 0.74 mM and Ferric Nitrate 22.3 mM in dilute acid and methanol) was pipetted to each tube. This was followed by the addition of 10 µl of calibrator/sample to respective tubes and then mixed. They were incubated at room temperature for at least five minutes and absorbance read at 480 nm. Chloride ion concentration (mEq/L) was calculated thus:

$$\text{Concentration (mEq/L)} = \frac{\text{Abs of Unknown} \times \text{conc. of standard.}}{\text{Abs of Calibrator}}$$

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2.5 Determination of Urea

2.5.1 Urea: Determination of urea concentration as described by (Randox kits).

Urea concentration was determined using the method of [19] as described in Randox kit.

Principle

Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured spectrophotometrically.

Urease

$$\text{Urea} + H_2O \longrightarrow 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenol} \longrightarrow \text{Indophenol (blue compound)}$$

Reagents (R)

R1: EDTA (116 mmol/L), Sodium Nitroprusside (6mmol/L),

Urease (1g/L)

R2: Phenol (120 mmol/L)

R3: Sodium hypochlorite (27 mmol/L), Sodium (0.14 N)

Procedure

Ten micro liter (10 µl) of distilled water (blank), standard calibrator (urea) and sample were added to three test tubes. This was followed by the addition of 100 µl of reagent to each of the test tubes. They were subsequently mixed and incubated at 37^{0C} for 10 minutes. The absorbance of the sample (A sample) and standard (A standard) against the blank was read at 546 nm

Calculation

$$\text{Urea Conc} = \frac{\text{Abs of sample} \times \text{conc. of standard (mmol/L or mg/dl)}}{\text{Abs of standard}}$$

1 mg of urea correspond to 0.467 mg of urea nitrogen.

2.5.2 Determination of creatinine

The serum creatinine was determined using the method of [19] as outlined in Randox kit.

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Principle

Creatinine in an alkaline solution reacts with picric acid to form a coloured complex. The amount of the coloured complex formed is directly proportional to the creatinine concentration.

Procedure

Two milliliter (2 ml) of the working reagent was mixed with 1ml of standard (creatinine) and incubated for 30 seconds. The same was done for the blood sample. The absorbance A1 of the sample and standard were taken at 492 nm. Exactly 2 minutes later, the absorbance A2 of the sample and standard were taken again. The serum creatinine was calculated thus:

$$\text{Serum Creatinine Conc.} = \frac{\Delta\text{Abs of Sample}}{\Delta\text{Abs of standard}} \times \text{standard conc. (mg/dl)}$$

A1 = absorbance 1

A2 = absorbance 2

$\Delta A = A2 - A1$ = change in absorbance (ΔA sample or ΔA standard)

This could either be in mg/dl or µ mol/L

2.6. Statistical Analysis

The data obtained were expressed as mean ± SD and were analysed using Statistical Product and Service Solutions (SPSS), version 20. Tests of statistical significance were carried out using both one-way and two-way Analysis of Variance (ANOVA). P values < 0.05 were considered Statistically significant.

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RESULTS

3.1 Percentage yield of ethanol leaf extract of *Polyalthia longifolia*

One thousand gram (1000g) of dried crude sample of *Polyalthia longifolia* leaf gave a percentage yield of 3.36%

3.2 Preliminary phytochemical screening

The results as depicted in Table1 shown that alkaloids and tannins occurred in high concentration, flavonoids and steroids occurs in moderate concentration, whereas terpenoids, glycosides and phenolic acid occurred in lowest concentration.

Table 1: Quantitative and Qualitative phytochemical screening of ethanol leaf extracts **of** *Polyalthia longifolia*

Phytoconstituents	Qualitative remarks	Concentration mg/100g
Alkaloids	+++	774.99±6.92
Flavanoids	++	646.25±10.44
Tannins	+++	879.62±0.58
Steroids	++	543.72±1.53
Terpenoids	+	36.38±2.02
Glycosides	+	36.48±0.48
Reducing sugar	+	94.83±0.52
Phenolic acid	+	33.45±0.55

Key: +++ = Present in high concentration
++ = Present in moderate concentration
+ = Present in lower concentration

3.3 Median Lethal Dose (LD_{50}) of the Ethanol Extract leaf *Polyalthia longifolia*

The ethanol extract of *Polyaltha logifolia* leaf (EPLL) did not produce any sign of toxicity or death during the observation periods of 24 hrs following oral administration up to a dose of 5000 mg/ kg. The absence of mortality and signs of any toxicity of the extract demonstrated that EPLL has a broader safety margin and indicating that the plant extract is not toxic.

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Table 2: Median lethal dose of ethanol extract of *Polyalthia longifolia* leaf

Phase I	Dose (mg/kg b.w)	Mortality Rate
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
PHASE II		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

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3.4 Effect of ethanol leaf extracts of *Polyalthia longifolia* on Castor oil-induced diarrhea in rat model.

Result in Table 3 showed a significant ($p < 0.05$) decrease in feacal droppings of groups treated with (200, 400, and 600 mg kg b.w of extract) when compared with positive control. Moreover, groups treated with 200 and 400 mg kg b.w of extract showed a significant ($p < 0.05$) reduction of feacal droppings at 5hrs when compared with 2, 3, and 4 hrs. In terms of protection, groups treated with 200, 400, and 600 mg kg b.w of extract showed a percentage inhibition of 67.60, 76.63, and 84.73 when compared with standard drug with percentage inhibition of 40.18 respectively.

Table 3: Effect of ethanol leaf extracts of *Polyalthia longifolia* on Castor oil-induced diarrhea in rats

Treated groups	**Wet feacal droppings at different time interval**					**Mean number of feacal droppings in 5hr**	**% Inhibition**
	1hr	2hr	4hr	3hr	5hr		
Group 1 Normal control	7.80 ± 0.84^{Ba}	7.2±0.44^{C} ^{d}	4.40 ± .89^{Cc}	3.00 ± 0.71^{Cc}	2.40±0.54 Cc	4.96±1.08	61.37
Group 2 Positive control	17.60 ± 1.34^{Ec}	15.00 ±1.58^{Ee}	12.60± 1.14^{Ee}	10.40 ± 0.51^{Ee}	8.60±1.51 Dd	12.84±2.62	_
Group 3 Standard control	12.80± .58^{Db}	10.60±1.14^{Dd}	8.40± 0.54^{Dd}	4.40 ± 0.54^{Dd}	2.20±0.83^{A} Cbc	7.68±0.72	40.18
Group 4 200mg/kg	9.80 ± 0.83^{Cc}	5.20 ± 0.37^{Bd}	3.00± 0.71^{Bd}	1.60 ± 0.24^{Bb}	1.20±0.44^{A} Bab	4.16±0.51	67.60
Group 5 400mg/kg	7.20±0.34^{Bb}	3.60±0.54 Ac	2.00±0.31^{A} Bab	1.40±0.54 ABab	0.80±0.37 Aa	3.00±0.42	76.63
Group 6 600mg/kg	4.60±0.89^{Ad}	2.60±0.24 Ac	1.60±0.54 Aa	0.60±0.24 Aa	0.40±0.24 Aa	1.96±0.43	84.73

Results are expressed in means ± SD. (n = 5)

Mean values having different upper case letters as superscripts are considered significant ($p < 0.05$) down the group.

Mean values having different lower case letters as superscripts are considered significant ($p <$

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0.05) across the period.

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3.5. Effect of ethanol leaf extract of *Polyalthia longifolia* on Gastrointestinal Motility in Rats models.

The Motility results as depicted in Table 4, showed that groups treated with 200,400 and 600 mg/kg b.w of extract showed a significant ($p < 0.05$) reduction in GIT motility when compared with the positive control. However, groups treated with 200,400 and 600mg/kg body weight of extract showed a percentage inhibition of 25.48%, 19.44%, 39.01% respectively. In terms of protection standard drug with percentage inhibition of 51.84% offered a better protection than treated extract with percentage inhibition of 25.48%, 19.44% and 39.01% respectively.

Table 4: Effect of ethanol leaf extract of *Polyalthia longifolia* on Gastrointestinal Motility in Rats.

Treatment Group	Gastrointestinal Transit	
	Peristaltic Index (%)	**% Inhibition**
Group 1 (Normal control)	72.66 ± 13.40ab	14.58%
Group 2 (Positive control)	85.06± 13.46^{c}	_
Group 3 (Standard control)	40.96 ± 10.15^{a}	51.84%
Group 4 (200mg/kg b.w)	63.38 ± 5.93^{abc}	25.48%
Group 5 (400mg/kg b.w)	68.52 ± 8.23^{bc}	19.44%
Group 6 (600mg/kg b.w)	51.87 ± 8.59^{ab}	39.01%

Results are expressed as Means ± SD (n = 5).

Mean values with different letters as superscripts down the column (the groups) are considered significant at $p < 0.05$

3.6 Effect of ethanol leaf extract of *Polyalthia longifolia* on electrolytes levels of castor oil induced diarrhea rats.

The results depicted in Table 5, indicated that groups treated with 200,400 and 600mg/kg body weight showed a significant ($P < 0.05$) increase in chlorine and sodium with corresponding decrease in bicarbonate and potassium level when compared with the positive control.

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Table 5: Effect of ethanol leaf extract of *Polyalthia longifolia* on electrolytes levels of castor oil-induced diarrhea in rats

Groups	Electrolyte Concentration (mmol/l)			
	Sodium	**Potassium**	**Bicarbonate**	**Chloride**
Group 1 (Normal control)	135.13±0.46^{c}	2.58±0.58^{ab}	24.84±2.11^{b}	97.22±0.58^{ab}
Group 2 (Positive control)	129.13±0.37^{a}	3.34±0.36^{c}	28.34±2.75^{c}	80.82±0.96^{a}
Group3 (Standard control)	138.1±0.46^{d}	3.57±0.39^{c}	26.42±1.34^{bc}	92.26±0.6^{b}
Group 4 (200mg/kg b.w)	132.38±0.14^{b}	3.04±0.6^{bc}	24.71±0.67^{b}	93.98±0.54^{c}
Group 5 (400mg/kg b.w)	141.04±0.44^{e}	4.50±0.45^{d}	27.26±0.79^{d}	100.32±0.12^{e}
Group 6 (600mg/kg b.w)	143.20±0.44^{f}	2.38±0.28^{a}	22 ± 0.87^{a}	104.69±0.48^{f}

Results are expressed as means ± SD (n = 5)

Mean values with different letters as superscripts down the group are considered significant at $p < 0.05$

3.7. Effect of ethanol leaf extract of *Polyalthia longifolia* on kidney function markers of castor oil-induced diarrhea rats

The results depicted from table 6, showed that ethanol leaf extract of *Polyalthia longifolia* exhibited a significant ($p < 0.05$) reduction in creatine and urea level when compared with the positive control. Moreover, groups treated with 200, 400and 600 mg/kg b.w in a dose dependent manner showed a significant ($p < 0.05$) reduction in creatine and urea when compared with positive control.

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Table 6: Effect of ethanol leaf extract of *Polyalthia longifolia* on kidney function markers of castor oil-induced diarrhea rats.

Treated group	Creatine (mg/dl)	Urea (mg/dl)
Group 1 (Normal control)	1.35 ± 0.12^{c}	3.13 ± 0.48^{b}
Group 2 (Positive control)	2.02 ± 0.52^{d}	4.96 ± 0.47^{d}
Group 3 (Standard control)	0.92 ± 0.06^{b}	2.38 ± 0.73^{a}
Group 4 (200mg/kg b.w)	1.51 ± 0.07	4.34 ± 0.19^{c}
Group 5 (400mg/kg b.w)	0.78 ± 0.07^{ab}	2.27 ± 0.25^{ab}
Group 6 (600mg/kg b.w)	0.53 ± 0.09^{a}	2.18 ± 0.17^{cd}

Results are expressed as means ± SD (n = 5)

Mean values with different letters as superscripts down the group are considered significant at $p < 0.05$

DISCUSSION

There is no scientific evidence to support the widespread usage of plant components for the treatment of numerous illnesses, particularly diarrheal sickness. The biological activity of plant extracts, which contain antispasmodic properties, delay intestinal transit, reduce gut motility, and boost water absorption, has been studied in a number of studies to support the use of medicinal plants' antidiarrheal properties [20]. A wide range of literature types revealed that plants with alkaloids, flavonoids, saponins, steroids, and tannins had been found to have antidiarrheal activity because of their antisecretory and antispasmodic effects on the gastrointestinal tract [21-22]. While tannins precipitate proteins, which lowers secretion and peristaltic motions, flavonoids have the capacity to limit intestinal motility and hydroelectrolytic secretions.[23] Moreover, tannin relaxes muscles by either stimulating the calcium pumping mechanism or lowering the intracellular Ca2+ inward current.[24]. Literature reports also indicated that flavonoids inhibit intestinal secretion

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induced by prostaglandin E2, saponins inhibit histamine release, terpenoids inhibit prostaglandin release, phenols reduce intestinal secretion and transit and have an astringent effect, and tannins have an antispasmodic and muscle relaxant effect. However, through reducing intestinal secretion and motility, all of these activities contribute to the suppression of diarrhoea.[25] The majority of these secondary metabolites were found in the ethanol extract of *P.logifolia* leaf. The plant's exact mode of action is still unknown, however these chemical components may be responsible for the plant extract's antidiarrheal activity, which also contributed to its antimotility, antisecretory, and ability to postpone the onset of diarrhoea. Since there were no signs of toxicity in the acute oral toxicity test at the limit dose of 5000 mg/kg in rat, the plant extract was deemed safe in terms of the acute toxicity test. Though there are many causes for diarrheal disease, the four main mechanisms behind the pathophysiology of diarrhoeas are (a) osmotic diarrhoea, which is caused by an increase in intraluminal osmolarity and a decrease in water absorption; (b) secretory diarrhoea, which increases the secretion of electrolyte; (c) deranged intestinal motility, which results in a decreased transit time [26]; and (d) inflammatory and infectious diarrhoea, which is caused by disruption of the intestinal epithelium of the intestine due to bacterial, viral, or protozoal pathogens and the immune response to inflammatory conditions in the bowel.[27]. Antimotility and antisecretory drugs are thought to be the cornerstone medications used in the management of diarrhoea because they reduce the pathophysiologic circumstances that lead to the development of diarrhoea.[28]. Moreover, castor oil releases ricinoleic acid, a metabolite that causes diarrhoea, it has been extensively utilised to induce diarrhoea in antidiarrheal investigations.[29]. Inflammation of the gastrointestinal mucosa causes prostaglandin to be released, which in turn increases gastrointestinal motility and electrolyte secretion, reducing the absorption of electrolytes from the colon and intestine. This mechanism is similar to the pathophysiologic processes that cause diarrhoea. This is how ricinoleic acid causes diarrhoea.[30]. It is well known that prostaglandins from the E series can cause diarrhoea in both humans and experimental animals. Therefore, it is thought that castor oil-induced diarrhoea is postponed by prostaglandin biosynthesis inhibitors.[31]. Conversely, loperamide, the typical antidiarrheal medication utilised as the positive control, is a synthetic opiate agonist that activates the m-opioid receptors in the large gut's myenteric plexus. [32]. The intestinal transit duration is increased by these physiological end effects, which also improve phasic colonic segmentation and inhibit peristalsis.[33] The inhibition of acetylcholine-mediated secretion is caused by loperamide's inhibitory effect on acetylcholine. Consequently, loperamide lowers daily faecal volume, lessens electrolyte and fluid loss, and may raise bulk density and viscosity in the stool.[34].

CONCLUSION:

The ethanol extract of the leaf of *P. logifolia* showed antidiarrheal activity on evaluation in animal models using Swiss albino Rat. The extract of the plant revealed a considerable delay in the beginning of diarrhea, reduced the frequency of wet feces and also endowed with significant antisecretory properties at all doses studied experimentally. Furthermore, the plant extracts demonstrated the antimotility action at higher concentrations. Even while additional research

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employing various antidiarrheal models and solvents is necessary, the study's results at this level confirmed.

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