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ANTIDIARRHOEAL EFFECT OF *CASUARINA EQUISETIFOLIA* ON EXPERIMENTAL ANIMALS

B. Anand Patil [1], A. Jyoti Admuthe [*2], R. Vikas Dhole [3] and S. Rani Dhole [4]

Department of Pharmacology [1], Rani Chenamma College of Pharmacy, 7/C, Bauxite road, BK Kangroli Industrial Area, Vaibhabvnagar, Belgaum - 590010, Karnataka, India.
Department of Pharmaceutics [2, 3], Annasaheb Dange College of D Pharmacy, Ashta, Sangli - 416301, Maharashtra, India.
Department of Pharmaceutics [4], Ashokrao Mane College of Pharmacy, Peth Vadgaon, Kolhapur - 416112, Maharashtra, India.

Keywords:

Castor oil, *Casuarina equisetifolia*, PGE2, CEME, Antidiarrhoeal

Correspondence to Author:
Mrs. Jyoti Avinash Admuthe

Assistant Professor,
Department of Pharmaceutics,
Annasaheb Dange College of D Pharmacy, Ashta, Sangli - 416301, Maharashtra, India.

E-mail: admuthe.jyotiadcdp@gmail.com

ABSTRACT: Current investigation was to evaluate antidiarrhoeal effect of *Casuarina equisetifolia*. Anti-diarrhoeal activity of methanol bark extract of *Casuarina equisetifolia* (CEME) studied on experimentally induced diarrhea in rats, castor oil-induced diarrhoea & enteropooling, meal transit time, and PGE2-induced entropooling. The CEME orally administered at doses 100, 200, and 400 mg/kg/atropine 0.1 mg/kg/ received control, respectively, were administered 30 min prior to the administration of castor oil. The CEME 100, 200 and 400 mg/kg/atropine 0.1 mg/kg/ received control to rats fed with as charcoal meal. Thirty minutes later, the intestinal distances moved by the charcoal were measured. The PGE2-induced enteropooling rats were treated with CEME orally at 100, 200, and 400 mg/kg/loperamide 2.5 mg/kg/1 ml of 5% v/v ethanol in normal saline, afterward PGE2 was administered orally to each rat, and intestine fluid volume was measured. The CEME 100, 200 and 400 mg/kg produced inhibition on the frequency of defecation by 33.89%, 42.66%, 59.64 %) and in diarrhoeic drops 35.83%, 43.06%, 49.72%. In PGE2-induced entropooling, intraluminal fluid accumulation reduced significantly. The charcoal meal transit time was significantly reduced, at CEME 100 mg/kg. Further at CEME 400 mg increased intestinal motility charcoal meal. The result shows that administration of CEME in rats causes antidiarrhoeal effect by reducing the total number of faeces, water content of faeces,inhibition of defecation, inhibition of diarrhoeic drops, and weight of intestinal content intestinal fluid volume and also increases the diarrhoeal onset time. From the above observation, it was concluded that CEME extracts exhibit significant dose-dependent antidiarrhoeal activity.

INTRODUCTION: Diarrhoea is associated with an increased frequency of bowel movements with the production of soft or watery stools.

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It may be defined as the passage of more than 300 ml of liquid faeces in 24 h [1]. Therapeutic measures of Diarrhoea may be grouped into the treatment of fluid depletion, shock, and acidosis.

Maintenance of nutrition drug treatment, an anti-diarrhoeal drug effective in non-infectious diarrhoea would inhibit secretion or promote absorption and produce some decrease in intestinal motility to permit a longer contact time of luminal fluids with epithelial cells.

The treatment of infectious diarrhoea may require the administration of antimicrobial agents. Anti-motility drugs act on bowel muscle to delay the passage of gut contents, so allowing time for more water to be absorbed. Anti-motility drugs should not be used for acute diarrhoea in children, especially babies, as there is a danger of causing paralytic ileus or respiratory depression. Oral rehydration salts are preferred for the treatment [2]. The most widely used prescription drug for diarrhoea is loperamide, which is chemically related to haloperidol and diphenoxylate, a weak analogue of meperidine [3].

Conventionally synthetic drugs are used to treat diarrhoea. Medicinal herbs are an indispensable part of the world due to their low cost, less side effects, easy access, and ancestral experience. In many literatures it has been reported that many indigenous plants in the traditional system of medicine are used for treating diarrhoea.

In the present study, the anti-diarrhoeal activity of methanol bark extract of *Casuarinae quisetifolia* (CEME) was carried out experimentally using induced diarrhea in rats, castor oil-induced diarrhoea, castor oil-induced enteropooling, charcoal meal transit time, and PGE-2 induced entropooling.

Results interpret that administration of CEME in rats causes antidiarrhoeal effect by a reduction in the total number of faeces, a total number of wet faeces, fresh weight of faeces, the water content of faeces, inhibition of defecation, inhibition of diarrhoeic drops, intestinal propulsion, the weight of intestinal content and volume of intestinal fluid, *etc*. and also increases the diarrhoeal onset of time.

Plant Profile:

- Title of plant: *Casuarina equisetifolia*.
- Family: Casuarinaceae
- Synonyms: She-oak, chabaku, sarvemara, janglisaru.
- Parts used: Bark
- Habitat: Srikalhasthi, Tirupathi, A sea-shore plant occurring wild on the shores at the East side of Bengal Bay from Chittagong southwards and Andaman's [4].

Chemical Constituents: The plants contains casuarin, σ-pyrocatechol,σ-gallocatechol, shikimic acid, quinic acid, sucrose, flavones, glycoside, cupressuflavone hydroquinone, ellagic acid, ß-sitosterol and other Phytosterol, catechin and its derivatives, gallic acid and methyl gallate, juglanin, atzelin and amino acids [5, 6].

Traditional uses: The decoction of bark and expressed juice of the seeds are used in the treatment of chronic diarrhoea and dysentery; the decoction of leaves is beneficial in the treatment of colic [7].

Pharmacological Actions: Antimicrobial activity, hepatoprotective activity, antioxidant [8-10]. *Casuarina equsetifoli*a has been found to reported activities like [11, 13].

- Antidiarrhoeal activity
- Hepatoprotective activity
- Antioxidant activity.
- Antimicrobial activity
- Antibacterial activity
- Antifungal activity

Another Plants Havinga Protective Effect on Diarrhoea [14, 17]:

- Dried latex of *Calotropis procera*
- Flowers *Argyreia speciosa*
- Leaves of *Aegle marmelos*
- Leaves of *Azadirachta indica*

MATERIAL AND METHODS:

Collection of Plant Material and Extraction: The *Casuarina equisetifolia* [CE] barks were collected from Alamati and Bagalkot districts, the region of North Karnataka. The *Casuarina equisetifolia* plant was authenticated at the Department of Botany, B.V.V. Sangha's Science College, Bagalkot. A voucher specimen 35/2011 was deposited in the same Institute: HSKCP/IAEC, Clear / 2010-11/1-12. The bark of *Casuarina equisetifolia* was shade-dried and uniformly powdered and subjected to hot continuous solvent extraction with petroleum ether

(40-60 °C) to defat, followed by methanol extraction. The solvent was completely removed by using a rotary flash evaporator and dried in a lyophilizer (Mini Lyotrap, LET Scientific Ltd, UK). These dried powdered extracts were formulated as a suspension in distilled water using 5% Tween-80 as a suspending agent. The percentage yield of extract *Casuarina equisetifolia* extracts in methanol solvent was found 14.2%, calculated in terms of dried weight.

$$\text{Yield} = \frac{\text{Weight of the extract}}{\text{Weight of the plant material}} \times 100 \ (\%)$$

All the chemicals were purchased from Hi-media, Mumbai and Sigma Chemicals Co., St Louis, USA, and were of analytical reagent grade.

Experimental Models for Screening Anti-diarrhoeal Activity: Studies on diarrhoea using animal models have contributed greatly to understanding the etiology and pathogenesis of disease and screening of anti-diarrhoeal agents. Diarrhoea can be produced experimentally in animals (mouse, rat, guinea pig, dog *etc*.) and are used for screening anti-diarrhoeal activity by the following methods:

Animals used: Albino Rats (wistar strain) weighing 200-250 gms of either sex and female albino mice weighing 20-25 gms were used in the present study. They were procured from HSK College of pharmacy animal house Bagalkot. The animals were allowed for acclimatization for ten d under laboratory conditions. They were housed in polypropylene cages and maintained at 27 ºC ± 2 ºC, relative humidity 65 ± 10% under 12 h light / dark cycle. The animals were fed with a rodent pellet diet (Gold Mohur Lipton India Ltd.) and water *ad libitum*. Animal ethical clearance for performing the experiments on animals was obtained from the Institutional Animal Ethical Committee (IAEC).

Determination of Acute Toxicity LD_{50}: The acute toxicity of methanol bark extracts of *Casuarinae qusetifolia* were determined in female albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed-dose OCED guideline No. 425; (Annexure-2) method of CPCSEA was adopted for toxicity studies [18, 19]. The test substance is directed in solitary by gavage utilizing a stomach tube or a reasonable intubation cannula. In the uncommon condition that a solitary portion is unimaginable, the portion might be given in more modest parts over a period not surpassing 24 h. Creatures ought to be abstained before dosing (*e.g.*, with the rodent, food yet not water ought to be retained for the time being; with the mouse, food, however not water, ought to be retained for 3-4 h). Following the time of fasting, the creatures ought to be gauged and the test substance directed. The abstained body weight is not set in stone, and the bodyweight determines the portion. After the substance has been controlled, food might be retained for 3-4 h in rodents or 1-2 h in mice. Where a portion is controlled in parts throughout some period, it could be important to give the creatures food and water contingent upon the length of the period.

The toxicity results are evaluated as follows (O = survival × = death)

The LD_{50} is less than the test dose (2000 mg/kg) when three or more animals die.

- XO XX
- OX XX
- XX OX
- XX X

If a third animal dies, conduct the main test.

Test five animals. The LD_{50} is greater than the test dose (2000 mg/kg) when three or more animals survive.

- OO OO
- OO XO
- OO OX
- OO XX
- XO XO
- XO OO/X
- OX XO
- OX OO/X
- XX OO

Creatures are noticed exclusively basically once during the initial 30 min after dosing, occasionally

during the initial 24 h (with exceptional consideration given during the initial 4 h), and d by d from there on, for a sum of 14 d, with the exception of where they should be eliminated from the examination and others consciously killed for creature government assistance reasons or are discovered dead. Notwithstanding, the length of perception must not be fixed unbendingly [20]. It must not really straighten out by the poisonous responses and season of beginning and length of recuperation period and may in this way be expanded when thought-about vital. The occasions at which indications of harmfulness show up and vanish are significant, particularly in case there is an inclination for poisonous signs to be delayed. All perceptions are deliberately recorded, with singular records being kept up with for every creature.

Extra perceptions will be essential if the creatures keep on showing indications of poisonousness. Perceptions should remember changes for skin and hide, eyes and mucous layers, respiratory, circulatory, autonomic and focal sensory systems, and somatomotor action and standard of conduct. Consideration should be coordinated to perceptions of quakes, spasms, salivation, loose bowels, dormancy, rest, and unconsciousness. Increased motor activity tremors, clonic convulsion, tonic convulsion, straubs reaction, piloerection musclespasm, catatonia, hyperthesia. Decreased motor activity ataxia, sedation, analgesia, anaesthesia, lacrimation, salivation, writhing, depression. The principles and criteria summarised in the humane endpoints guidance document should be taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress should be humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded as precisely as possible.

Castor Oil-induced Diarrhoea in Rats: The method previously described was used for the experiment. The animals were randomized divided into 6 groups, each group containing six animals. Rats of either sex weighing 200-250 g were fasted for 18 h and randomly assigned to six groups of six animals. One hour after extract administration, each animal will receive 1 ml of castor oil orally and then be observed for defecation up to 4 h.

The presence of characteristic diarrhoeal dropping will note in transparent plastic dishes placed beneath individual cages [21].

- Group I: Normal (2% v/v aqueous Tween 80)
- Group II: Control (Castor oil + 2% v/v aqueous Tween 80)
- Group III: Castor oil and Standard drug (5 mg/kg loperamide)
- Group IV: Castor oil and 100mg/kg of methanol extract.
- Group V: Castor oil and 200mg/kg of methanol extract.
- Group VI: Castor oil and 400mg/kg of methanol extract.

Each animal was kept individually in a cage, filter paper, 30 min after the extract/Loparamide treatment, each animal was administrated 1 ml of castor oil orally, following the organization of castor oil, the creatures were put in independent enclosures, and the onset time (The time between oil administration and appearance of first diarrhoeal drop). Observed for defection continue up to 6 h on pre-weighted (MO) filter paper placed beneath the individual perforated rat cages. The used paper was reweighted (M1) if it had wet faeces was evaluated as (M1- MO) g. Finally, the filter paper was exposed in the laboratory to dry over a period of 14 h and was reweighted again ((M2).

The water content of the faeces was calculated as (M2- M1) g. The onset time, the mean number of defecation, the number of wet spots, the fresh weight, and the water content were recorded. After analysis of the means of the various parameters per group, the percentages of inhibition of defecation and diarrhoeic drops were evaluated as indicated by [22].

$$\text{Inhibition of defecation (\%)} = \frac{Mc - Md}{Mc} \times 100$$

Mc: Mean number of defecation caused by castor oil; Md: mean number of defecation caused by drug/extract.

$$\text{Inhibition of diarrhoeic drops} = \frac{Mc - Md}{Mc} \times 100$$

Mc: Mean number of drops caused by castor oil; Md: Mean number of drops caused by drug/extract.

All the statistical comparisons between the groups were made through One Way Analysis of Variance (ANOVA) and followed by Dunnett's Multiple Comparison test. The P <0.05 was regarded as significant using Graph Pad Prism 5.01 Software (Graph Pad Software, San diego, CA, USA). The data expressed are Mean ± standard error of the mean (S.E.M.)

Castor Oil-induced Enter Pooling: In this method, rats were deprived of food and water for 18 h, and animals were randomly selected per group six rats. After extract administration 30-min later, 1 ml of castor oil is the administrator for each animal. After 30 min administration of castor oil to each rat was sacrificed and the end of the small intestine tied. From the pylorus to caecum were dissected out, and its contents were collected in 2.5 ml syringe whose tip was sealed, and the volume of intestinal fluid and weight of small intestinal contents were measured [23].

- Group I: Control (1 ml of charcoal meal)
- Group II: Standard (2.5 mg/kg loperamide)
- Group III: 100 mg/kg of methanol extract
- Group IV: 200 mg/kg of methanol extract
- Group V: 400 mg/kg of methanol extract

Mc - Md % Inhibition of intestinal fluid76 (ml) = × 100 Mc

Mc: Intestinal fluid (ml) caused by castor oil; Md: Intestinal fluid (ml) caused by drug/extract.

Gastrointestinal Motility Test: Rats will be kept on fasting for 18 h and placed in six cages containing six mice. Each animal will receive orally 1 ml of charcoal meal (3% deactivated charcoal in 10% aqueous Tragacanth).

Immediately after extract administration and 30 min later, each animal will be sacrificed. The intestinal distance moved by the charcoal meal from pylorus was cut, measured, and expressed as a % of the distance from the pylorus to the caecum for each animal [24].

- Group I: Normal (2% v/v aqueous Tween 80)
- Group II: Control (1 ml of charcoal meal)
- Group: Standard (0.1 mg/kg atropine, i.p.)
- Group: 100 mg/kg of methanol extract
- Group: 200 mg/kg of methanol extract
- Group: 400 mg/kg of methanol extract

The Intestinal Propulsion % was evaluated as Indicated by [25]: Distance moved by the suspended charcoal head.

Intestinal propulsion % = Whole length of small intestine

PGE2-induced Enter Pooling: In this method, rodents were denied food and water for 18 h and were set in five groups with six animals in every cage.

The animals were separated into 5 groups of albino rats of wistar albino rats, weighing 200-250 g, segregated into control, positive control, and test groups of containing six experimental animals immediately after administration of the extract.

PGE2 will be given orally to each rat. After 30 min consumption of PGE2, rats will be sacrificed, and afterward entire length of the digestive system from the pylorus to caecum will be cut out. Intestinal sub stance weight and the volume were calculated [26].

- Group I: Normal (5% ethanol)
- Group II: Control (100 µg/kg PGE2)
- Group III: 100 mg/kg of methanol extract
- Group IV: 200 mg/kg of methanol extract
- Group V: 400 mg/kg of methanol extract

Mc-Mtx 100 % Inhibition of intestinal fluid (ml) = Mc

Mc: Intestinal fluid (ml) caused by PGE2; Mt: Intestinal fluid (ml) caused by drug/extract.

RESULTS:

Castor Oil-Induced Diarrhoea: Castor oil 1 ml per animal produced diarrhoea that lasted for 6 h. All the rats produced copious diarrhoea.

The results obtained in the evaluation of the CEME at doses 100, 200, 400, and loperamide 2.5 mg/kg were shown in **Table 1**.

TABLE 1: EFFECT OF *CASUARINA EQUISETIFOLIA* METHANOL EXTRACT ON CASTROL OIL-INDUCED DIARRHOEA

S. no.	Groups	Onset time (min)	Total no faeces	Total no Wet faeces	Fresh weight of faeces (g)	Water content of faeces	Inhibition of defection (%)	Inhibition of diarrhoeic drops (%)
I	Control	58.17 ± 1.79	12.67 ± 0.666	4.333 ± 0.557	1.410 ± 0.173	0.8767 ± 0.1413	0	0
II	Loparamide 2.5 mg/kg	-	-	-	-	-	100 %	100 %
III	CEME 100 mg/kg	66.16 ± 1.346€	9.000 ± 0.966€	2.667 ± 0.210€	1.320 ± 0.236¥	0.725 ± 0.093¥	33.89 %	35.83 %
IV	CEME 200 mg/kg	68.48 ± 2.240£	7.000 ± 0.894£	2.167 ± 0.307£	0.845 ± 0.057€	0.518 ± 0.039€	42.66 %	43.06 %
V	CEME 400 mg/kg	105.30 ± 2.508≠	5.000 ± 0.730≠	2.000 ±0.365 ≠	0.824 ± 0.061€	0.508 ± 0.034€	59.64 %	49.72 %

All values are mean ± SEM (n=6) One-way analysis of variance test (ANOVA) followed by Dunnette's multiple comparison test. Normal and treated groups are compared to control groups. Whereas ¥ Non-significant, ≠P<0.001, £P<0.01, €P<0.05.

Onset of Time: The control, loperamide (STD), and CEME effect on the onset of time were shown in Table no.1 and **Fig. 1A.** m The administration of castor oil caused 58.17 ± 1.79 min to produce diarrhoea. The administration of CEME 100,200 and 400 mg/kg significantly (P<0.05-0.001 *vs*. Gr-II) increased the 66.16 ± 1.346 min,68.48 ± 2.240 min, and 105.30 ± 2.508 min respectively, onset of diarrhoeal time when compared to control. However, 400 mg/kg has increased the diarrhoeal time to 105.30 min compared to 100,200 mg/kg of CEME treated rats. This suggested that CEME shows dose-dependent activity on this model.

Total Number of Faeces: The control, loperamide (STD) and CEME effect on the total number of faeces were shown in **Table 1** and **Fig. 1B.** The administration of castor oil caused significantly (P< 0.01), 12.67 ± 0.666 produces the number of faeces. The administration of CEME 100,200 and 400 mg/kg significantly (P<0.05-0.001 *vs* Gr-II) reduced the 9.000 ± 0.966, 7.000 ± 0.894, 5.000 ± 0.730, respectively. A total number of faeces when compare to control. However, 400 mg/kg has reduced the total number of faeces 5.000 ± 0.730 compared to 100,200 mg/kg of CEME treated rats.

Total Number of wet Faeces: The control, loperamide (STD) and CEME effect on the total number of wet faeces were shown in **Table 1** and **Fig. 1C.** The administration of castor oil caused 4.333 ± 0.557 to produce a number of wet faeces. The administration of CEME 100,200 and 400 mg/kg significantly (P<0.05-0.001 *vs.* Gr- II) reduced the number of wet faeces 2.667 ± 0.210, 2.167 ± 0.307, 2.000 ± 0.365 respectively, Total number of wet faeces when compared to the control. However at 400 mg/kg has reduced the total number of wet faeces 2.000 ±0.365 when compared to 100, 200 mg/kg of CEME treated rats.

Fresh Weight of Faeces (gms): The control, loperamide (STD), and CEME effect on fresh weight of faeces were shown in **Table 1** and **Fig. 1D.** The administration of castor oil caused 1.410 ± 0.173 to produce the weight of faeces. The administration of CEME 100, 200 and 400 mg/kg significantly (P<0.05-0.001 *vs* Gr-II) reduced 1.320 ± 0.236, 0.845 ± 0.057, 0.824 ± 0.061, respectively fresh weight of faeces when compare to control. However at 400 mg/kg has reduced the fresh weight of faeces 0.824 ± 0.061 when compared to 100, 200 mg/kg of CEME treated rats.

Water Content of Faeces: The control, loperamide (STD) and CEME effect on water content of faeces were shown in **Table 1** and **Fig. 1E.** The administration of castor oil caused 0.8767 ± 0 to produce water content of faeces. The administration of CEME 100, 200 and 400 mg/kg significantly reduced (P <0.05-0.001 *vs.* Gr-II) to the 0.725 ± 0.093, 0.518 ± 0.039, 0.508 ± 0.034, respectively water content of faeces when compare to control. However, 400 mg/kg has reduced the water content of faeces 0.508 ± 0.034 compared to 100, 200 mg/kg of CEME treated rats.

Inhibition of Defecation: The CEME 100, 200, and 400 mg/kg produced inhibition on the frequency of defection by 33.89%, 42.66%, and 59.64%, and response shown in **Fig. 1F.**

Inhibition of Diarrheic Drops: The CEME produced inhibition of diarrhoeic drops by 35.83, 43.06 and 49.72% and response is shown in **Fig. 1G.**

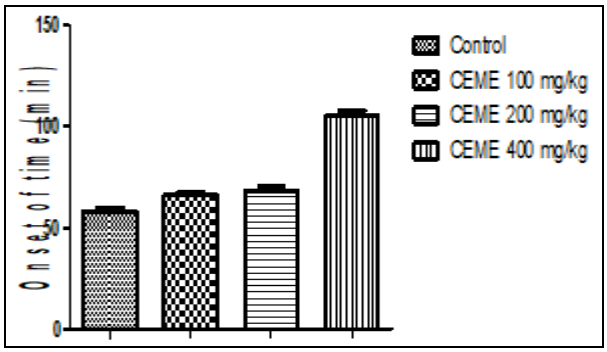

FIG. 1A: ONSET OF TIME

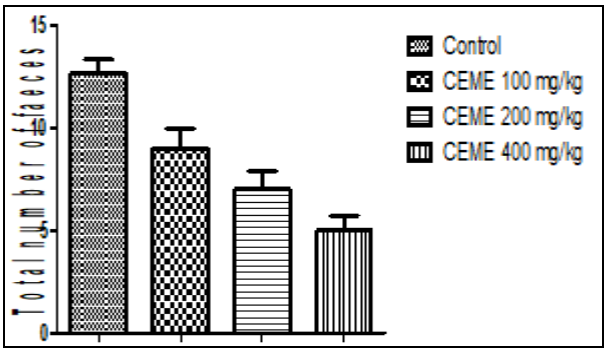

FIG. 1B: TOTAL NUMBER OF FEACES

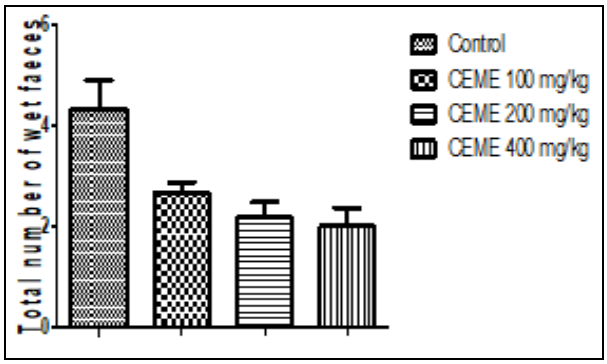

FIG. 1C: TOTAL NUMBER OF FEACES

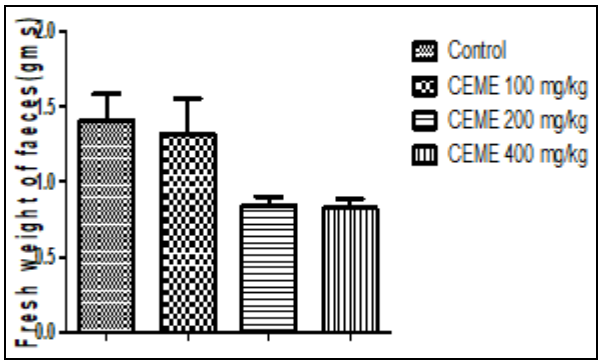

FIG. 1D: FRESH WEIGHT OF FEACES

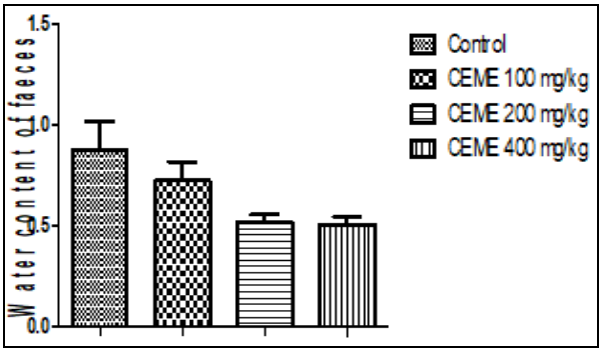

FIG. 1E: WATER CONTENT OF FEACES

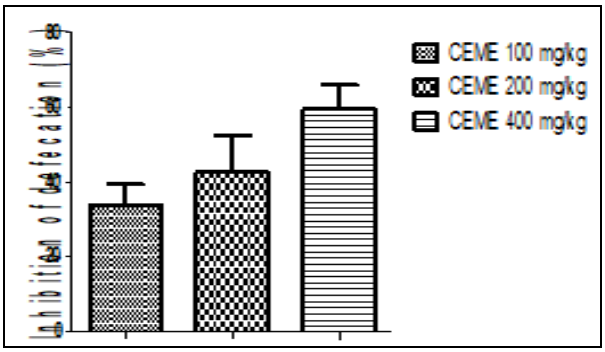

FIG. 1F: INHIBITION OF DEFECATION

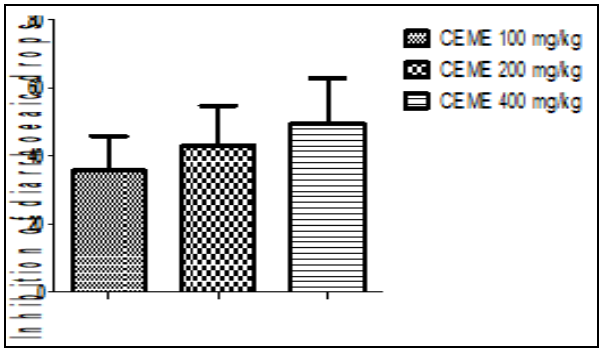

FIG. 1G: INHIBITION OF DIARRHOEAIC DROPS

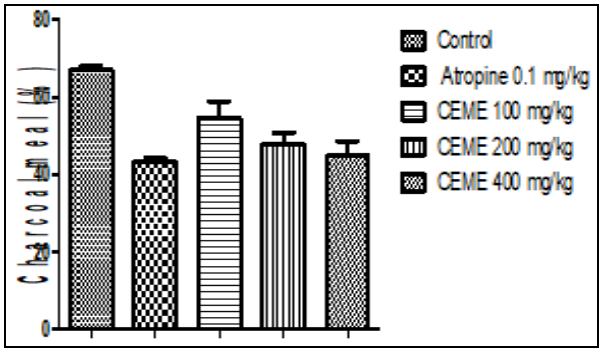

FIG. 1H: MEAN DISTANCE TRAVELLED BY CHARCOAL

FIG. 1: EFFECT OF *CASUARINA EQUISETIFOLIA* METHANOL EXTRACT ON CASTROL OIL-INDUCED DIARRHOEA(A): ONSET OF TIME, (B): TOTAL NUMBER OF FEACES, (C): TOTAL NUMBER OF FEACES, (D): FRESH WEIGHT OF FEACES, (E): WATER CONTENT OF FEACES, (F): INHIBITION OF DEFECATION, (G): INHIBITION OF DIARRHOEAIC DROPS, H): MEAN DISTANCE TRAVELLED BY CHARCOAL

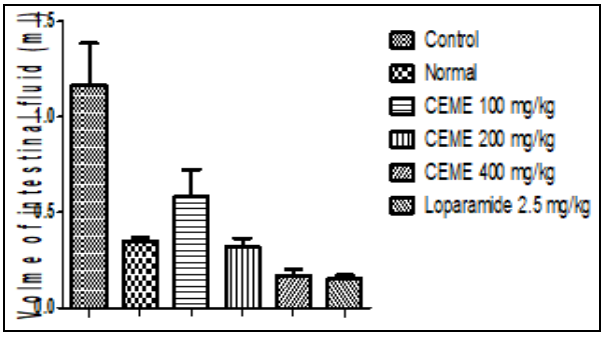

FIG. 2A: VOLUME OF INTESTINAL FLUI

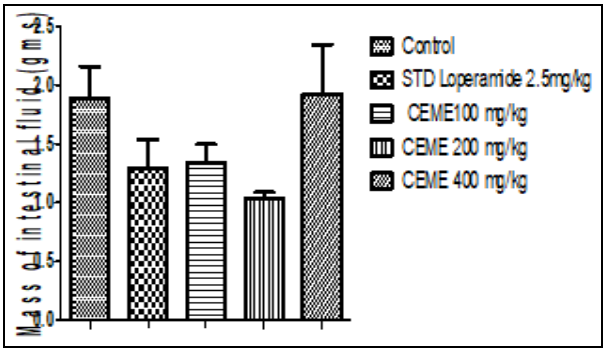

FIG. 2B: MASS OF INTESTINAL FLUI

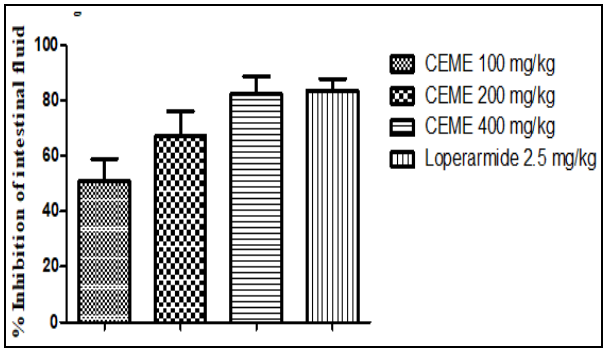

FIG. 2C: PERCENT INHIBITION OF FLUID

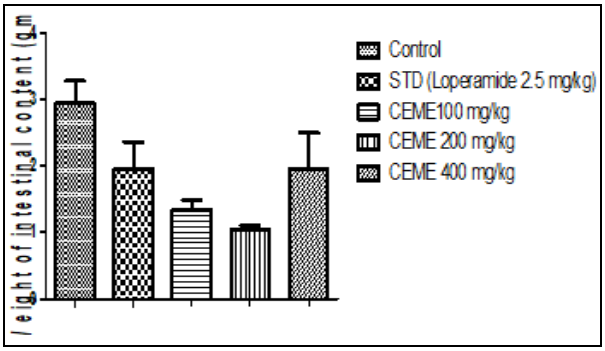

FIG. 3A: WEIGHT OF INTESTINAL CONTENT

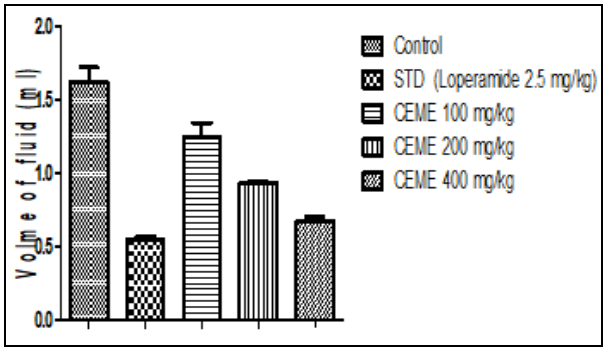

FIG. 3B: VOLUME OF INTESTINAL FLUID

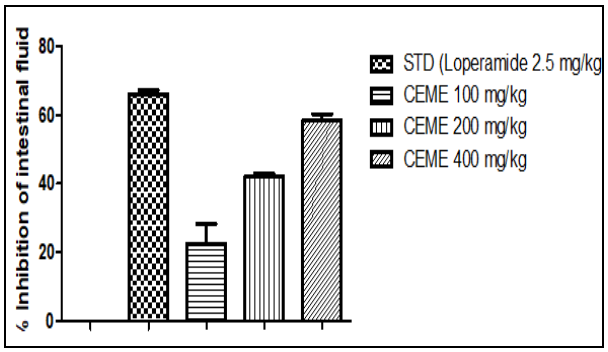

FIG. 3C: INHIBITION OF INTESTINAL FLUID

FIG. 3: EFFECT OF *CASUARINAE QUISETIFOLIA* METHANOLIC EXTRACT ON CASTOR OIL-INDUCED ENTEROPOOLING; (A): WEIGHT OF INTESTINAL CONTENT, (B): VOLUME OF INTESTINAL FLUID, (C): INHIBITION OF INTESTINAL FLUID

Castor Oil-induced Enteropooling: The results of castor oil-induced enteropooling of controls treated of CEME and standard loperamide (2.5 mg/kg) were shown in Table 4 and **Fig. 3A, B** & **C**. The weight of intestinal content and volume of intestinal fluid has been significantly decreased in CEME of 100, 200, and 400 mg/kg ($P< 0.01$ - 0.001) in (Gr III, IV, and V) when compared to control (Gr I). Corresponding to % of inhibition 22.56%, 42.13%, 58.28% and loperamide 66.13%

respectively. Further, CEME 400 mg/kg was found to be more inhibition. Similarly volume of intestinal fluid significantly (P< 0.001 vs. control) decreased in CEME 100 mg/kg, 200 mg/kg , 400 mg/kg and loperamide 2.5 mg/kg (P< 0.001) when compared to control. Whereas there is a significant decrease in loperamide 2.5 mg/kg and CEME 400 mg/kg when compared to control (Gr. I).

Gastrointestinal Motility Test: The controls treated of charcoal meal of small intestine were shown in **Table 2 and Fig. 1H**. In control Gr-I, the charcoal meal traversed 66.95 of the total length of the small intestine in control (Gr-I).

The CEME 100, 200 and 400 and atropine (0.1 mg/kgi.p) decreased the intestinal propulsion by (P<0.001) 45.28 ± 3.476, (P<0.05) 54.42 ± 4.983, (P<0.01) , 48.48 ± 3.834, and 43.16 ± 0.9626 % (P<0.001 vs. Gr-I) which is equivalent to 28.81%, 23.95%, 16.75% and 33.46 % of intestinal propulsive inhibition relative to control respectively, when compared (Gr-II, III, IVvs.Gr-I .

TABLE 2: EFFECT OF *CASUARINA EQUISETIFOLIA* METHANOLIC EXTRACT ON THE GASTROINTESTINAL MOTILITY TEST IN RATS

S. no.	Groups	Intestinal length (cm)	Mean distance travelled by charcoal meal	% of inhibition
I	Control	99.00 ± 1.673	66.75 ±1.051	-
II	Atropine 0.1 mg/kg	102.2 ± 2.134	43.16 ± 0.9626$^{\neq}$	33.46 ± 1.796
III	CEME 100 mg/kg	102.4 ± 2.764	54.42 ± 4.983$^{€}$	16.75 ± 5.794
IV	CEME 200 mg/kg	105.1 ± 3.333	48.48 ± 3.834$^{£}$	23.95 ± 4.117
V	CEME 400 mg/kg	104.5± 1.285	45.28 ± 3.476$^{\neq}$	28.81 ± 4.638

All values are mean ± SEM (n=6) One-way analysis of variance test (ANOVA) followed by Dunnette's multiple comparison test. Normal and treated groups are compared to control groups. Whereas ¥ Non-significant, ≠P<0.001, £P<0.01, €P<0.05.

PGE2-induced Entropooling: The controls treated of PGE2 were shown in **Table 3** and **Fig. 2a, b, c.** The PGE2 induced a significant (P<0.001vs. normal) increased rat's intestinal fluid volume when it is distinct from the control group of animals on only ethanol in normal saline. The CEME 100, 200, 400 and loperamide (2.5 mg/kg). Significant (P<0.001) decreased the (P<0.001vs. control) when compared (Gr- IV, V, VIvs. Gr-I). However, 400 mg/kg produced a significant decrease in the fluid volume of the rat intestine (P<0.001 vs. control). The mass of intestinal fluid also measured decreased in intestinal fluid with CEME 100, 200, 400 mg/kg, and loperamide appreciably hindered PGE2 entropooling induced in rat animals.

TABLE 3: EFFECT OF *CASUARINAE QUISETIFOLIA* METHANOLIC EXTRACT ON PGE2 - INDUCED ENTEROPOOLING

S. no.	Groups	Weight of intestinal content (g)	Volume of intestinal fluid (ml)
I	Control	1.88 ± 0.27	1.167 ± 0.218
II	Normal	1.85 ± 0.32	0.350 ± 0.022
III	Loperamide (2.5mg/kg)	1.87 ± 0.36$^{¥}$	0.1500 ± 0.223
IV	CEME 100 mg/kg	1.34 ± 0.15$^{¥}$	0.583 ± 0.140$^{€}$
V	CEME 200 mg/kg	1.03 ± 0.05$^{¥}$	0.316 ± 0.0477$^{£}$
VI	CEME 400 mg/kg	1.92 ± 0.43$^{¥}$	0.166 ± 0.0333$^{\neq}$

All values are mean ± SEM (n=6) One-way analysis of variance test (ANOVA) followed by Dunnette's multiple comparison test. Normal and treated groups are compared to control groups. Whereas ¥ Non-significant, ≠P<0.001, £P<0.01, €P<0.05.

TABLE 4: EFFECT OF *CASUARINA EQUISETIFOLIA* METHANOLIC EXTRACT ON CASTOR OIL-INDUCED ENTEROPOOLING

S. no.	Groups	Weight of Intestinal content (g)	Volume of intestinal fluid (ml)	% inhibition of Intestinal fluid accumulation
I	Control	2.94 ± 0.33	1.61 ± 0.10	-
II	Loperamide(2.5mg/kg)	1.95 ± 0.40$^{¥}$	0.54 ± 0.01$^{\neq}$	66.13 ± 1.14$^{\neq}$
III	CEME 100 mg/kg	1.34 ± 0.14$^{€}$	1.24 ± 0.09$^{£}$	22.56 ± 5.83$^{\neq}$
IV	CEME 200 mg/kg	1.04 ± 0.05$^{£}$	0.93 ± 0.01$^{\neq}$	42.13 ± 0.77$^{\neq}$
V	CEME 400 mg/kg	1.96 ± 0.53$^{¥}$	0.67 ± 0.03$^{\neq}$	58.28 ± 1.92$^{\neq}$

All values are mean ± SEM (n=6) One-way analysis of variance test (ANOVA) followed by Dunnette's multiple comparison test. Normal and treated groups are compared to control groups. Whereas ¥ Non-significant,≠P<0.001,£P<0.01,€P<0.05.

DISCUSSION: The effect of the *Casuarina equisetifolia* methanolic bark extract on experimentally animal-induced diarrhoea was estimated using diarrhea which is induced by castor oil, Charcoal meal transit time, and entropooling induced by PGE2 in the rat. Traditionally to treat diarrhoea the bark is used as water decoction overnight. Methanol is a strong polar solvent considered to extract most plant secondary constituents. Castor oil-induces diarrhoea by causing increased secretion of fluid and electrolytes into the lumen of the bowel by intestinal mucosa, resulting in fluid accumulation and a watery luminal content that flows rapidly through the small and large intestines [27].

Castor oil is 90% ricinoleate, and its diarrhoea inducing property is known to be due to its active metabolite ricinoleic acid, which diminishes Na+ and Cl- permeability in the intestine [28, 29]. Furthermore, ricinoleic acid can also lead to the release of endogenous prostaglandins, which play an important role in the modulation of GIT, stimulate motility and secretion and cause local diarrhoea irritant, which irritates the gastrointestinal mucosa resulting in enhancement of intestinal motility [30, 32]. *Casuarina equisetifolia* 400 mg/kg, Loperamide 2.5 mg/kg shown highest inhibition on the frequency of defection by 59.64%, andin diarrhoeic drops 49.72 and loperamide (2.5 mg/kg) completely prevented the diarrhoea. Further, a significantly ($P<0.001$), prolonged the time of onset of diarrhoea and significantly ($P<0.001$) reduced the total number faeces. The fresh number weight and water content significantly decreased ($P<0.05$), showing a dose-dependent activity.

The *Casuarina equisetifolia* extract might; act through stimulation of reabsorption of water from the intestinal lumen or anti-prostaglandin activities that contribute to the pathophysiological function in the gastrointestinal tract [84]. In the evaluation of intestinal transit, atropine sulphate was used as a standard drug. Atropine is known to inhibit intestinal transit, probably due to its anticholinergic effect [33]. CEME reduced the intestinal propulsive movement in the charcoal meal treated model at all the test doses (100, 200, and 400 mg/kg body weight). It is logical since atropine sulphate is pure compared to the extracts, which are mixtures of many compounds. Studies on activated charcoal showed that it prevents the absorption of drugs and chemicals into the system by avidly adsorbing them on the surfaces of the charcoal particles [34]. Activated charcoal was used in the gastrointestinal motility test to find out the effects of these extracts on the peristaltic movement. The results show that these extracts suppressed the propulsion of charcoal meal (probably in the same way as atropine sulphate), thereby increasing the time for absorption of water and electrolytes. Castor oil administration to rats stimulates small intestinal transit, as shown in **Table 2.** Oral administrations of *Casuarina equisetifolia* extract inhibited the small intestinal charcoal meal motility and reduction in the propulsive movement of the small intestine with extract or atropine, which further supports the antidiarrhoeal activity.

Agents that reduce intestinal motility and secretion are known to possess antidiarrhoeal activity.35 The chemical constituents of CEME are casuarin, σ-pyrocatechol, σ-gallocatechol, shikimic acid, quinic acid, sucrose, flavones, glycoside, cupressuflavone hydroquinone, ellagic acid, ß-sitosterol, and other Phytosterol, catechinand its derivatives, gallic acid and methyl gallate, juglanin, atzelin and amino acids [36, 37]. Tannins are responsible for protein denaturation producing protein tannate, which reduces secretion from the intestinal mucosa. CEME also contains tannin, which may produce antisecretory activity.

Administration of PGE2 to rat caused significantly ($P<0.001$vs. control) increased volume in intestine fluid accumulation. The CEME at 200 and 400 mg/kg significantly inhibited the PGE2- induced enteropooling showing **Fig. 2A** & **B**. with the CEME 400 mg/kg showing inhibition 82.27% having a better activity comparable with that of the loperamide (2.5 mg/kg) 83.71%. Prostaglandin contributes to the pathophysiological function in gastrointestinal tract [38]. The *Casuarina equisetifolia* was used as an astringent and to treat diarrhoeadysentery and Antimicrobial, tannins, other plant metabolite possess antidiarrhoeal activity in deferent experimental animals model [39, 40]. Tannins are known to reduce secretion and make the intestinal mucus resistant through the formation of protein tannate.

It may be possible that the tannins present in the extract could contribute to the observed effects. In our study, the antidiarrhoeal activity of *Casuarina equisetifolia is* similar to Loperamide/ Atropine (standard antidiarrhoeal agent). The potency of *Casuarina equisetifolia* is less than the standard antidiarrhoeal agent due it could be a result of its being crude in nature.

The standard antidiarrhoeal agent, however, is used in its pure form. The extracts significantly inhibited the castor oil-induced intestinal fluid accumulation (enter pooling) and weight of intestinal content. It has been shown that castor oil causes motility and secretory diarrehoea.

The mechanism involved has been associated with dual effects on gastrointestinal motility and water and electrolyte transport (decreasing Na+ and K+ absorption) across the intestinal mucosa. These conditions tend to suggest that the CEME extracts of 100, 200, and 400 mg/kg reduced diarrhoea by increasing reabsorption of electrolytes and water or by inhibiting induced intestinal accumulation of fluid, just as loperamide.

Loperamide acts by decreasing the transit velocity and increasing the capacity of the intestines to retain their fluids [41]. Perhaps a similar mechanism could explain the action of the *Casuarina equisetifolia*. So, it is possible that the tannins and flavonoids content of the plant may be responsible for the antidiarreals activity of the *Casuarina equisetifolia*.

CONCLUSION: The result shows administration of CEME in rats causes an antidiarrhoeal effect by increasing the diarrhoeal onset of time and by a reduction in the total number of wet faeces, fresh weight of faeces, water content of faeces, inhibition of defecation, inhibition of diarrhoeic drops and reduced intestinal propulsion.

In the case of castor oil-induced enter pooling and PGE2- induced enteropooling, there is a reduction in the weight of intestinal content and volume of intestinal fluid. From the above observation, it was concluded that CEME extracts exhibit significant dose-dependent antidiarrhoeal activity. Therefore, *Casuarina equisetifolia* constituent's tannins and flavonoids of the plant may be liable for the antidiarrhoeal activity.

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