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Original Research Article

INVESTIGATE AND EVALUATE OF ANTI DIARRHOEAL ACTIVITY OF CAPPARIS ZEYLANICA USING ANIMAL MODEL

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Abstract: Diarrhoea remains a major global health problem, particularly in developing countries, contributing significantly to morbidity and mortality, especially among children and the elderly. Although several synthetic antidiarrhoeal drugs are available, their prolonged use is often associated with adverse effects and limited accessibility. Therefore, the exploration of safe, effective, and affordable plant-based alternatives is of great importance. Capparis zeylanica, a medicinal plant widely used in traditional systems of medicine, has been reported to possess various pharmacological properties, including antimicrobial, antioxidant, and anti-inflammatory activities. However, its antidiarrhoeal potential has not been sufficiently validated scientifically. The present study was undertaken to investigate and evaluate the antidiarrhoeal activity of Capparis zeylanica using experimental animal models. The methanolic extract of Capparis zeylanica was prepared and subjected to preliminary phytochemical screening to identify bioactive constituents. Acute toxicity studies were performed to establish the safety profile of the extract. The antidiarrhoeal activity was assessed in laboratory animals using standard models such as castor oil-induced diarrhoea, gastrointestinal transit (charcoal meal test), and enteropooling assays. The results demonstrated that the extract of Capparis zeylanica significantly reduced the frequency of defecation, volume of intestinal contents, and intestinal motility in a dose-dependent manner when compared to the control group. The observed antidiarrhoeal effect may be attributed to the presence of phytoconstituents such as flavonoids, tannins, alkaloids, and saponins, which are known to exhibit antisecretory, antimotility, and antimicrobial activities. ***In conclusion***, the findings of this study provide scientific evidence supporting the traditional use of Capparis zeylanica in the management of diarrhoea. The plant exhibits promising antidiarrhoeal activity and could serve as a potential source for the development of new, safe, and effective herbal antidiarrhoeal formulations.

Keywords: Capparis zeylanica, Antidiarrhoeal activity, Medicinal plants, phytochemical screening, Animal models, Castor oil-induced diarrhoea, gastrointestinal motility, Herbal medicine.

INTRODUCTION

1.1 Diarrhoea

Diarrhoea is commonly defined as the recurrent and excessive expulsion of loose or watery liquid from the intestines [1]. This phenomenon occurs due to heightened motility of gut. It could be either acute or chronic. Diarrhoea results in the production of watery intestinal output as a consequence of inadequate fluid absorption. While the root responsible for diarrhoea must be provisional, the frequency of illness occurrence has remained consistent, and this treatment often proves ineffective in cases where patients experience substantial stool production [2]. The utilization of herbal remedies for the cure of diarrhoea is a prevalent in numerous regions of India and Africa. Plants, which are plentiful in nature and widely embraced by the population, serve as cost-effective substitutes for

traditional treatments. Therefore, it is crucial to seek out and accurately document herbs that possess anti-diarrheal properties [3]. Scientific identification of plant, encompassing presentation of photographs when feasible, geographical distribution or cultural or ethnomedical utilization (alongside other potential ethnomedical applications), the phytoconstituents present in the plant, and the attributed mechanism(s) of action of the plant [4–6]. Maximum children who succumb to diarrheal diseases reside in less developed countries, with Africa accounting for 78 percent of these fatalities. Ethiopia ranks fifth globally in terms of mortality rates for pneumonia and diarrhea across the globe [7].

The prevalence of diarrhoea among children under the age of five in Ethiopia was found to be 13% within a two-week period. After lower respiratory infections, it is the second major reason of death [8–9]

The medicinal plants L. camara, Calpurnia aurea, Croton marcostachyus, and Echinops kebercho are utilized by traditional healers in Ethiopia to cure diarrhea, however the anti-diarrheal properties of L. camara have not been scientifically investigated. L. camara is known by various common names worldwide, including as black sage, cuasquito, angel lip, flowered sage, shrub verbena, white sage, and wild sage [10-12]. The native names for this in Ethiopia are Yewef kollo (in Gedeoffa and Amharic languages)

The Enaro belongs to the Maale ethnic community, while the Michi-Charo belongs to the Shako ethnic group [13-16].

Nevertheless, it is important to note that this particular application has not yet been subjected to scientific assessment [17]. Nevertheless, it is highly chances aforementioned acts is present, as there have been several publications indicating the potential anti-diarrheal benefits of L. camara leaf extract [18–19].

Diarrhoea commonly serves as an indicator of intestinal problems, precipitated by a diverse array of bacterial, viral, and parasitic (protozoa and helminths) microorganisms, such as E. coli, Vibrio cholerae, Shigella species, and other others [20].

1.2 Capparis zeylanica L.

Stragglers, branchlets adpressed tomentose. dimensions leaves exhibit an oval shape with a mucronate apex, truncate base, & pubescence, with dimensions ranging from 7 to 9 cm and 5 to 6 cm. petiole measures around 1 cm in length & possesses a significant pubescence, characterized by the existence of small, paired, recurved stipular spines. Blooms exhibit a vertical alignment above axillary region, characterized by a width ranging from 3 to 4 cm. buds display a white hue and possess a compact density of pubescence.The length of the pedicels ranges from 2 to 4 cm, & the exhibit pubescence. Stamens are abundant, elongated, white, & might develop a brown color. The length of the gyn androphore is of equal or greater magnitude than that of the filaments. Ovary measures 2.5 mm and its form are ellipsoid. [21].

MATERIAL AND METHODS

2.1Experimental Work:

Authentication of *Capparis zeylanica* roots:

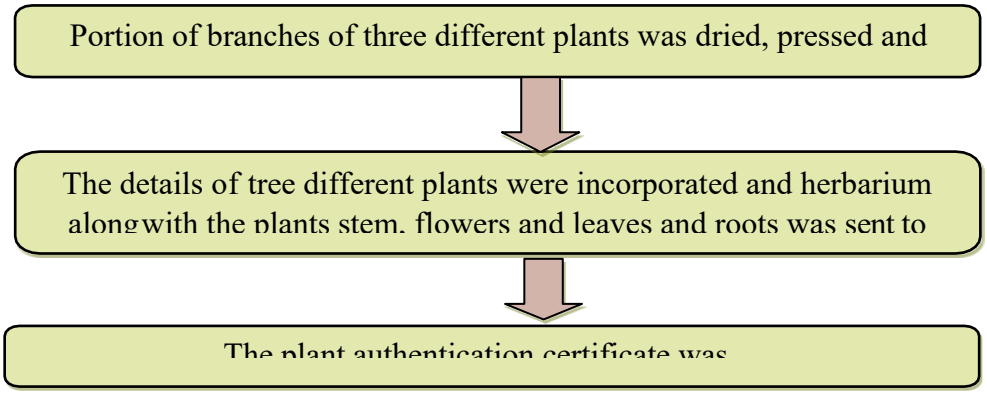

Figure No. 1: Schematic representation for the process of preparation of plant herbarium and authentication of *Capparis zeylanica*

2.2 Preparation of leaves and roots powder: A fresh water was used to eliminate dirt and subsequently rinsed with distilled water. The foliage of Capparis zeylanica was subjected to shadow drying under ambient conditions. & were powdered. dry powder (25g) was exhaustively extracted with suitable

solvent using three different soxhlet apparatus (14 extraction cycles) at different temperatures. & were powdered. dry powder (25g) was exhaustively extracted with suitable solvent using three different soxhlet apparatus (14 extraction cycles) at different temperatures.

2.3 Optimization of solvents and temperature for the preparation of different plant extracts: Extraction solvent was optimized by extracting the *Capparis zeylanica* highest polarity solvents Methanol using soxhlet apparatus. methanolic extract was subjected to phytochemical screening. Solvent amount and extraction temperature were optimized to get the optimum condition for maximum yield.Solvent concentration was optimized by varying amounts of solvent used (50, 75, 100, 150 ml) and the temperature at which at which the extraction was to be carried out was optimized by varying temperature conditions 25, 30, 40, 50 ºC of heating mantle of soxhlet apparatus. Time/cycles was optimized at 10, 12, 14 and 16 extractions.'

2.4Phytochemical screening: Preliminary qualitative phytochemical screening of methanolic extract was done by using standard phytochemical screening methods. Change in color was noted for the result.

2.5 TLC: Step involve in performing TLC of three different plant extracts:
Capparis zeylanica
Pre-coated TLC plate: Silica gel- G
Sample application: Capillary was immersed in solution & the sample was applied to the Capillary at a location about 2cm from one end of the plate to the thin layer plate. The place was dried out by air.
Chamber saturation: For aegirine detection, a TLC chamber was saturated with mobile phase toluene: ethyl acetate: formic acid (6: 4: 0.1 v/v) at room temperature (20 ºC - 25 ºC). The mobile phase was poured into the chamber, which was then covered with a lid and left to saturate for 30 minutes.
Chromatogram Development: The plate was kept in the chamber after the chamber had been saturated and the sample had been spotted on plate. Otherwise, Instead of undergoing chromatography, the spotted material would dissolve in a solvent pool. level at bottom of chamber was kept below level of spot put to plate. Solvent was allowed to flow roughly a third of the way around the silica plate.
Visualization: Plates were removed & were examined visually & in iodine chamber and after the retention factor was calculate by following formula.**[22]**

2.6 FTIR: The Fourier transform has a value of 5.7. The emergence of infrared spectroscopy can be ascribed to its appropriateness in transforming organic data into a perceptible spectrum. At different wave numbers, each functional group exhibits one or more distinct peaks and a specific frequency range. The aforementioned technology possesses the capacity to perceive vibrations, quantify the displacement of chemical bonds, and measure the extent of bending and stretching. The FTIR spectrum of sample was obtained using FTIR Spectro photo meter (Make:) 10 mg of extract was directly placed on the sample compartment (made up of selenium coated diamond) and the spectrum was obtained in the range of 4000-650 cm^{-1} at a resolution of 8 cm^{-1} for the characterization of chemical functional groups. The FTIR data gives the estimation of compound present in the extract. FTIR helps in determination of compatibility between excipients and active ingredients as presence of all the peaks at respective places indicates active ingredient's compatibility with excipients. However, minor shifting or change in intensity of peak is acceptable due to mixing with other components **[23]**.

2.7Animals Studies
A. Selection of animals
Wistar albino rats experiment utilised a sample with a weight ranging from 150 to 350 grimes' comprehensive examinations was conducted on animals to confirm absence of any pathological diseases. Animal facility provided by Himalayan Institute of Pharmacy for the experiment.
B. Animal approval
The research was carried out subsequent to gaining clearance from the IAEC, and the experimental protocols adhered to the parameters outlined in proposal No HIP/IAEC/2022//06.

C. Selection of animals
The study encompassed the recruitment of Wistar albino rats exhibiting a weight distribution ranging from around 150 to 350 grimes' comprehensive examination was conducted on the animals to confirm the absence of any pathological diseases. The rodents were acquired from the animal facility at NIPER Mohali for the purpose of this investigation.

D. Charcoal meal test- gastrointestinal motility
The rats, with a weight range of 150 to 350 grammes, were subjected to an 18-hour fasting period during which they were provided with free access to both water & food. At first, the animals were given castor oil orally. Control group, referred to as Group I, was administered oral normal saline solution. The experimental group, denoted as Group II, was administered loperamide orally at a dose of 3 mg/kg. Group III and IV received test medication (EEPA) at dosages of 250 and 500 mg/kg,

respectively, in this trial.

Group I received an oral administration of a normal control, which consisted of a saline solution & 1ml/kg charcoal meal. Group II participants received oral administration of Loperamide at dosages of 3mg/kg & 1ml/kg charcoal meal, in accordance with normal protocol. Group III participants received oral administration of EEPA at dosages of 250mg/kg and 1ml/kg charcoal meal. Group IV participants received oral administration of EEPA at dosages duration of one hour, each rat was administered a 5% suspension of charcoal at a dose of 1ml/kg. Animals were killed by cervical dislocation after a duration of 30 minutes, and subsequently dissected.

The whole gastrointestinal system, spanning from the pylorus to the caecum, was surgically excised and later arranged in a longitudinal manner on a conventional white paper sheet. The researchers recorded.

2.8 Castor - oil induced diarrhea

The cause of this is the activity of ricinoleic acid, which is produced as a result of the degradation of oil. This acid induces changes in the transport of water and electrolytes, resulting in hypersecretory responses. Wistar rats weighing between 200 and 350 grams were subjected to an 18-hour fasting phase, during which they had access to unlimited food. The animals were individually housed in cages and subsequently divided into four groups, each including six individuals.

Group I acted as the control group and was administered normal saline orally at a dosage of 1ml/kg.
Group II was given the normal medication loperamide as a 3 mg/kg oral dose.
Methanolic extract at doses of 250 and 500 mg/kg were administered orally to Group III and IV.
Group I: Oral administration of a normal control.
Group II: Administration of loperamide at a dosage of 3mg/kg orally.
Group III: Oral administration of EEPA at a dosage of 250mg/kg.
Group IV: Administrator of EEPA (500mg/kg) orally

2.9 Castor oil enter pooling

The study included the administration of an 18-hour fasting period to Wistar rats with weights ranging from 200 to 350 grams. Throughout this time, the rats were given free access to both water and food. The animals were individually housed in enclosures and then divided into four cohorts, with each cohort including six individuals. The control group, referred to as Group I, received oral administration of normal saline at a dose of 1ml/kg.

Group I, a normal control was administered orally.
Group II was administered the standard medicine loperamide orally at a dosage of 3 mg/kg.
Group III and IV were orally fed methanolic extract at dosages of 250 and 500 mg/kg. In
In Group II, loperamide was administered orally at a dose of 3mg/kg. Participants in Group III were administered EEPA orally at a dose of 250mg/kg.
Group IV: Administration of oral EEPA at a dosage of 500mg/kg.

One hour later, an oral administration of castor oil at a dosage of 0.1 ml per rat was conducted. All rats were euthanized by overdosing with di ethyl ether 30 minutes after being administered castor oil. A whole segment of the intestine, spanning from pylorus to caecum, was surgically removed. Contents were then gathered in a measuring cylinder & their volume was determined.

2.10 Magnesium sulphate induced diarrhea

The study included the administration of an 18-hour fasting period to Wistar rats with weights ranging from 200 to 350 grams. Throughout this time, the rats were given free access to both water and food. The animals were individually housed in enclosures and then divided into four cohorts, with each cohort including six individuals. Group I acted as the control group and was administered normal saline orally at a dosage of 1ml/kg. Group II was administered the conventional medicine loperamide as an oral dose of 3 mg/kg. After duration of one hour, a rat was subjected to an oral dose of magnesium sulfate at a dosage of 1 ml. As a result, the animals were subsequently, the specimens were transferred into separate cages that were lined with white blotting paper. Following a 4-hour duration, combined quantity of dry and moist faeces expelled was quantified and subsequently compared to the control group

Figure No. 2: Characteristics of *Capparis zeylanica*

RESULT

3.1 Morphological studies of fresh *Capparis zeylanica* plant: According to morphological investigations of the Capparis zeylanica is a botanical species that exhibits robust growth on recently cut foliage. Height of the plant varies between 12 & 15 meters, although its trunk is quite short. Bark is dense, smooth, & in a granular condition. Branches have a broad & uneven arrangement, whereas the lowest branch has a downward trajectory. Spines of juvenile suckers have a stiff & linear morphology. Botanical structure of deciduous plant consists of a sequence of 3 to 5 leaflets that are oval in shape, pointed, and possess shallow toothing. Length & width of these leaflets range from 4 to 10 cm and 2 to 5 cm, respectively. Petiole of terminal leaflet is elongated and may be seen either alone or in clusters.

3.2 Phytochemical Screening of different obtained plant extracts: By performing identification tests on extracts, several phytochemicals present in extract were checked, such as alkaloids, glycosides, tannin, phenolic compound, carbohydrate, flavonoids, amino acid, and terpenoids. Tables No.7 summaries the results of phytochemical screening.

Table No.1: Phytochemical screening of Methanol extract

Sr. No.	Phytochemical tests	Observation	*Capparis zeylanica*
1.	**Alkaloid**		
1.1	Hager's test	Yellow precipitates	+
1.2	Wagner's test	Reddish or brown color precipitates	++
1.3	Mayers test	Cream color precipitates	+
2.	**Saponin glycosides**		
2.1	Foam test	1 cm foam	+
3.	**Cardiac glycosides**		
3.1	Killer kills ani test	The presence of cardiac glycoside is indicated by the junction of two liquid layers and the emergence of a blue green color in the top layer.	-
3.2	Legal test	Appearance of pink color	-
4.	**Tannins a nd phenolic compounds**		
4.1	Lead acetate test	Yellow colored precipitate	+
4.2	Ferric chloride test	Greenish black color	-
5	**Carbohydrate**		
5.1	Molish test	A reddish violet ring is observed at the interface between two layers.	-
5.2	Bendict test	Green, yellow and red precipitate	-
5.3	Felhing Test	1st yellow than brick red color precipitates	-
7.	**Terpenoids**		
7.1	Salkowski test	Reddish brown color	++

8.	**Flavonoids**		
8.1	Lead acetate test	Yellow color precipitate	+

++: high content, +: moderate, -: negative

3.3 Physicochemical evaluation of different plant extract:

To evaluate the percentage yield, density, pH, total ash, and Rf for TLC, physicochemical analysis of several plant extracts was performed. Below Table No.2 shows data on physicochemical features of several plant extracts.

Table No. 2: Physicochemical characteristics of different obtained plants extract

Sr. No	**Parameter**	***Capparis zeylanica* Observation (Mean ± SD)**
1.	Percentage yield	3.94±0.03
2.	Density	2.56±0.02
3.	pH	5.21±0.01
4.	Total ash	2.50±0.01
7.	TLC	0.71±0.01

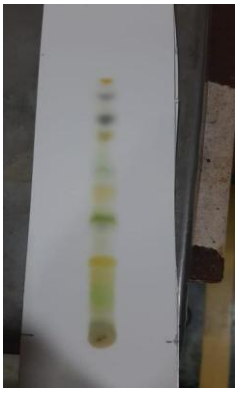

Figure No.3: TLC of *Capparis zeylanica* plant extract

3.4 FTIR Spectral analysis: FTIR spectroscopy was employed to analyze the pure methanolic plant extract of *Capparis zeylanica* were scanned from 400 to 4000 cm^{-1}. The spectra is shown is below figure

3.4.1 Fourier transform Infra-red (FTIR) spectroscopy of *Capparis zeylanica* extract: FTIR spectrum predicted the structure of Carotene (Fig.). FTIR spectra of extract showed peak around 2929.38 cm^{-1} due to C-H stretching , 1997.85 cm^{-1} due to C=O stretching, 1611.55 cm^{-1}due to C=C stretching and around 1248.53 cm^{-1} due to C-O stretching.

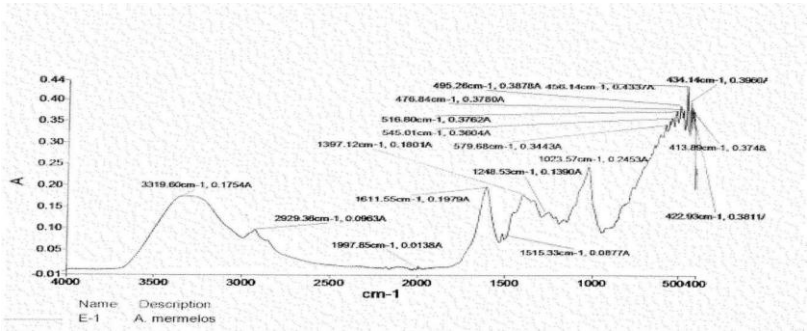

Figure No .4: FTIR spectra of plant extract

Table No.3: Effect of EEPA on charcoal meal induced hyperperistalsis

Group	Mean length of small intestine (cm)	Mean distance travelled by charcoal (cm)	Peristalsis index (%)
Controlled	85.5 ± 0.85	58.1 ± 0.75	68.05 ± 0.62
Castor oil	87.3 ± 0.87	62.5 ± 0.89	82.19 ± 0.49[a]
Castoroil+Loperamide	88.5 ± 0.56	59.8 ± 0.80	49.84 ± 0.61[b]
Castor oil+250 mg/kg	86.8 ± 0.89	63.2 ± 0.91	58.69 ± 0.81 [b]
Castor oil+500 mg/kg	85.1 ± 0.79	54.5 ± 0.79	56.11 ± 0.59 [b]

Activity related with methanolic leaf extract of Capparis zeylanica was evaluated via the use of charcoal meal hyperperistalsis test. In compared to rats treated with castor oil, administration of EEPA extract at dosages of 250 & 500 mg/kg demonstrated a statistically significant (P<0.01) anti-diarrheal effect. Among two doses examined, it was observed that 500 mg exhibited most pronounced anti-diarrheal effect (56.14±0.76), while ordinary castor oil showed a comparatively lower level of activity (49.94±0.62). Charcoal infused with castor oil exhibited a higher coverage area. Extracts exhibited a notable anti-diarrheal effect (p<0.01). Graphical representation of activity is seen in below figure.

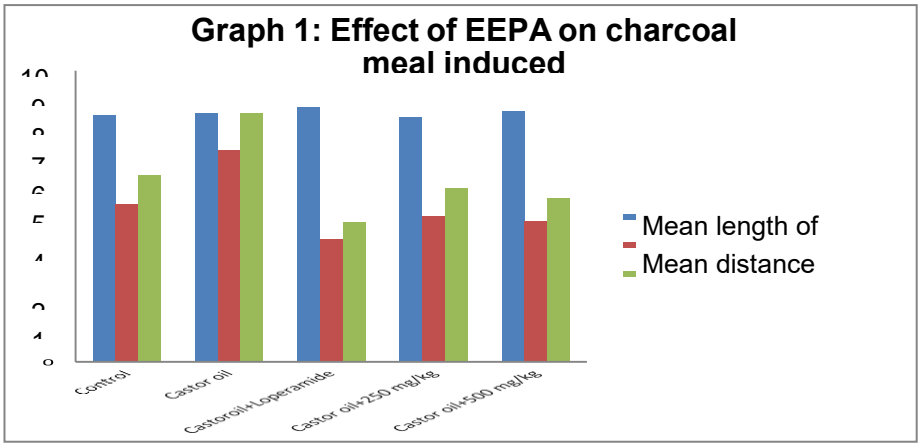

Figure No. 5: Effect of EEPA on charcoal meal induced hyperperistalsis

Table No.4: Effect of EEPA on anti-diarrhoea effect on castor oil induced diarrhoea in wistar rats

Group	Water intake (ml)	Feed intake (gm)	Weight of fecal matte in 4 hours (gm)
Control	16.7 ± 0.6	15.49 ± 0.6	4.01 ± 0.05
Castor oil	20.8 ± 0.7[a]	18.82 ± 0.5[a]	6.60 ± 0.03[a]
Castoroil+Loperamide	17.6 ± 0.9[c]	15.84 ± 0.5[c]	4.54 ± 0.06[c]
Castor oil+250 mg/kg	19.5 ± 0.8[c]	16.93 ± 0.8 [b]	5.68 ± 0.05[b]
Castor oil+500 mg/kg	19.4 ± 0.7[c]	16.87 ± 0.7 [c]	5.01 ± 0.07[c]

Measures undertaken & data analyzed indicate a notable anti-diarrheal efficacy. Rats that were administered castor oil indicates a statistically significant rise (p<0.01) in both water and food intake, as well as an increase in faecal

matter weight, as compared with. Rats that were administered methanolic leaf extract of Capparis zeylanica exhibited a statistically significant decrease ($p<0.01$) in the weight of faecal matter consumed, including both water and feed consumption, as compared to rats treated with castor oil. Administered at a dosage of 500 mg/kg had the most significant anti-diarrheal effect (5.01 ± 0.06) in the treated rats, despite the fact that the extract at a lower dosage of 250 mg/kg had a relatively lesser impact (5.68 ± 0.04) compared to conventional drug Loperamide. Visual depiction of action is shown in below figure.

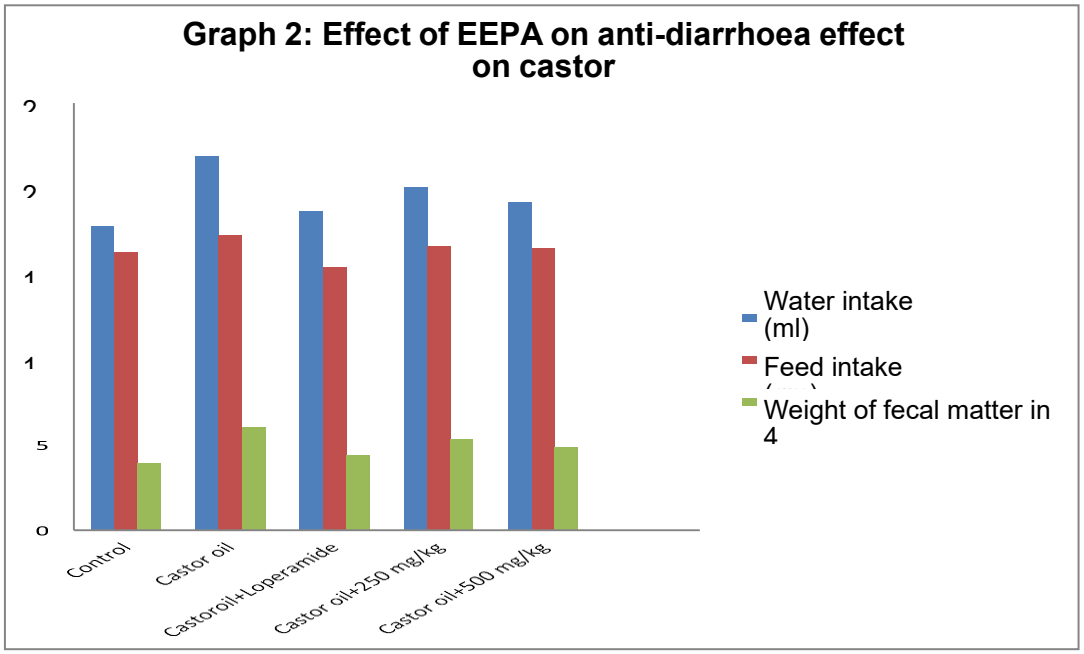

Figure No.6 *Effect of EEPA on anti-diarrhoea effect on castor oil induced diarrhoea in Wistar rats*

Table No.5: Effect of EEPA on Castor oil enter pooling in Wistar rats

Group	Weight of intestinal content (gm)	% Inhibition in weight intestinal content (gm)
Control	4.8 ± 0.8	----
Castor oil	7.08 ± 0.6[a]	----
Castoroil+Loperamide	5.18 ± 0.8[c]	27.14
Castor oil+250 mg/kg	5.79 ± 0.4[c]	16.12
Castor oil+500 mg/kg	5.45 ± 0.5[c]	21.95

Notable anti-diarrheal efficacy. Rats that were administered castor oil showed a statistically significant rise ($p<0.01$) in both water and food intake, as well as an increase in faecal matter weight. Rats that were administered methanolic leaf extract of Capparis zeylanica exhibited a statistically significant decrease ($p<0.01$) in the weight of faecal matter consumed, including both water and feed consumption. Extract administered at a dosage of 500 mg/kg had the most significant anti-diarrheal effect (5.01 ± 0.06) in the treated rats, despite the fact that the extract at a lower dosage decrease ($P<0.01$), with decreases of 5.79 ± 0.4 & 5.45 ± 0.5, respectively. Furthermore, a reduction of 16.12% & 21.95% in weight of intestinal content was seen in rats subjected to castor oil treatment. rats who received a combination of castor oil & standard medication loperamide had most significant degree of inhibition (21.95%) in relation to weight of their intestinal content. anti-diarrheal impact of EEPA extract was shown to be dependent on dosage, with most pronounced reaction recorded at a dosage of 500mg/kg. This response was comparatively lower than response reported in rats treated with loperamide. A visual representation of activity is shown. In below figure.

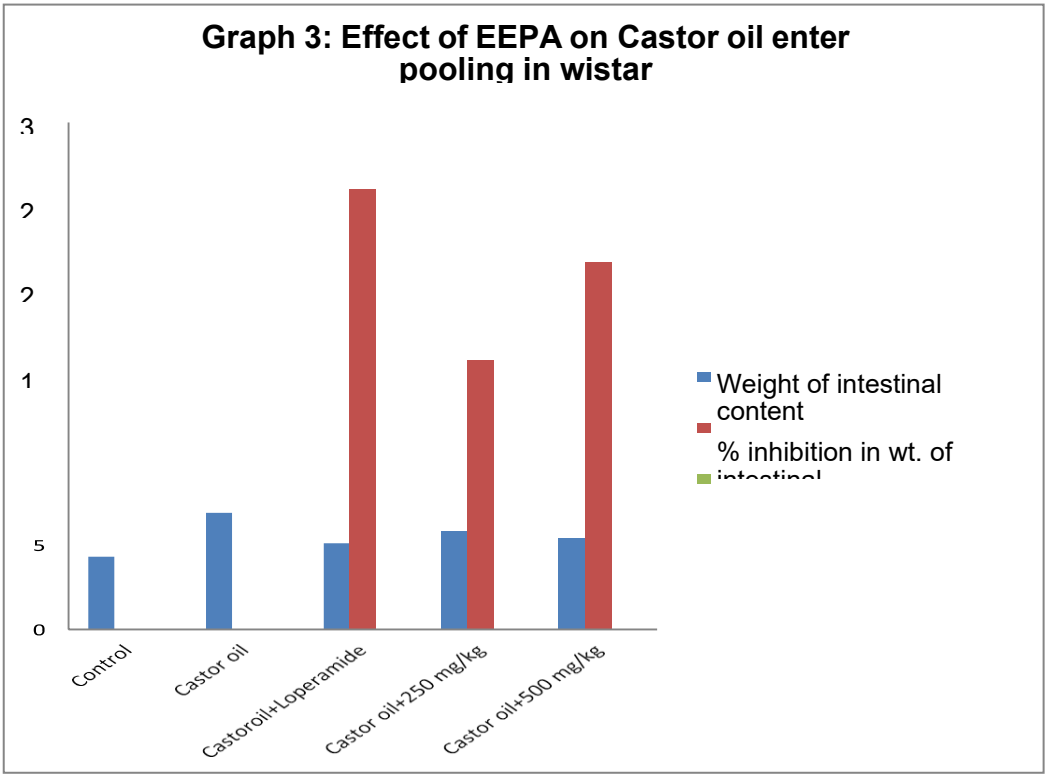

Figure No.7: Effect of EEPA on Castor oil enter pooling in wistar rats

Table No. 6: Effect of EEPA on anti-diarrhoea effect on Magnesium sulphate induceddiarrhoea in Wistar rats

Group	Water intake (ml)	Feed intake (gm)	Weight of fecal matter in 4 (gm)
Control	17.9 ± 0.56	16.25 ± 0.5	4.02 ± 0.02
Magnesium sulphate ($MgSO_4$)	20.8 ± 0.6[a]	17.74 ± 0.2[a]	5.44 ± 0.10[a]
$MgSO_4$+Loperamide	18.42 ± 0.7[c]	15.63 ± 0.6[c]	4.68 ± 0.12[c]
$MgSO_4$+250 mg/kg	19.72 ± 0.9[c]	16.85 ± 0.6 [b]	5.67 ± 0.10[b]
$MgSO_4$+500 mg/kg	19.27 ± 0.6[c]	16.41 ± 0.4 [c]	5.02 ± 0.05[c]

Primary objective that research potential anti-diarrheal effects methanolic leaf extract obtained from Capparis zeylanica. This was achieved by inducing diarrhea in Wistar rats using magnesium sulfate. To evaluate anti-diarrheal efficacy, many parameters were analyzed, such as water consumption, feed consumption, & fecal matter weight. A statistically significant was seen in rats with magnesium sulfate-induced diarrhea, as compared to rats without any dietary limitations. In compared to rats treated with magnesium sulfate, administration of EEPA at dosages of 250 & 500 mg/kg led to a significant decrease in water consumption. After a 4-hour period, weight of the fecal matter decreased significantly (5.02 ± 0.05) when given a dose of 500 mg/kg, compared to treatment with EEPA (5.67 ± 0.10). but it was seen to be reduced in comparison to animals administered the standard medicine

loperamide. Representation of activity is visually shown in figure no.8

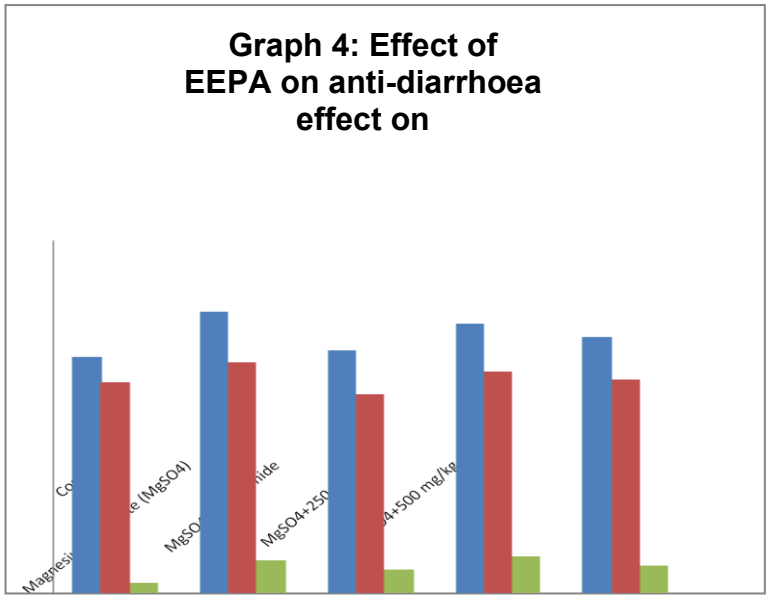

Figure No.8: Effect of EEPA on anti-diarrhoea effect on Magnesium sulphate induced diarrhoea in wistar rats

CONCLUSION

Present study, entitled "Evaluation of the anti-diarrheal properties of methanolic leaf extract of Capparis zeylanica," conducted an inquiry to determine its effectiveness in treating diarrhea in animal models. Presence of tannins in extract may be responsible for its efficacy. Results of this study provide empirical evidence supporting possible therapeutic efficacy of methanolic leaf extract derived from Capparis zeylanica in the management of diarrhea & its related symptoms.

Further investigation is required to evaluate the effectiveness of Capparis zeylanica methanolic leaf extract in the treatment of diarrhea using other experimental models. Procedure of extracting active component, assessing its effectiveness in an experimental model, exploring its mechanisms of action will finally result in creation of innovative medications.

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