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Formulation And Evaluation Of Antidiarrhoeal Activity Of Ethanolic Leaf Extract Of Prunus Avium And Cedrus Deodara In Swiss Albino Mice

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Abstract

Traditionally, the leaves of *Prunus Avium and Cedrus Deodara* have been used to treat diarrhoea. Its effectiveness hasn't been scientifically proven, though, thus far. This study looked at the antidiarrheal properties of Prunus Avium and Cedrus deodara leaf extracts in mouse models. In models of Swiss Albino mice, the antidiarrheal efficacy of ethanolic leaf extracts of *Prunus Avium and Cedrus Deodara* was investigated. The extract's effects at various dosages were contrasted with those of common medications and mouse groups used as negative controls. While the number of wet feces was significantly reduced by the extract at all tested dosages, a dose of 600 mg/kg of castor oil-induced diarrhoea showed a considerable reduction in the frequency of defecation. Additionally, at all tested doses, it demonstrated a noteworthy and dose-dependent decrease in the mouse model's intestinal content; the observed findings at 400 and 600 mg/kg were superior to those of loperamide, the standard medication. At any of the tested doses, a substantial antimotility impact was not seen, nevertheless. These findings indicate that the ethanolic leaf extracts of *Cedrus deodara and Prunus avium* exhibited antidiarrheal properties.

Keywords: - Prunus Avium, Cedrus Deodara, formulation and evaluation of extract

1. INTRODUCTION

Diarrhoea is a medical disorder marked by an elevated frequency and fluidity of an individual's bowel movements. Diarrhoea is the term used to describe a condition where the water content in the stool is between 60-90% (more than 90%). Millions of individuals perish annually in developing nations. Children are particularly vulnerable to this illness. [1,2] The initial symptoms of dehydration typically manifest as irritability and reduced skin elasticity, which can then escalate to reduced urine output, pallor, increased heart rate, and less responsiveness as the condition worsens. The condition can manifest as either acute or chronic, with varying degrees of severity ranging from mild to potentially fatal. Herbal therapy is a traditional method of healthcare that has been practiced since ancient times. Plants has the capacity to produce a diverse range of chemical compounds, which serve crucial biological purposes and provide protection against predators such as insects and fungi. The utilization of plants for medical purposes appears to have been derived from the careful observation of animals and subsequent experimentation.[3,4]

Around 12,000 compounds of this nature have been extracted from plants. Several pharmacological medicines commonly used by physicians, such as opium, digitalis, quinine, and aspirin, have a well-established background as herbal remedies. Phytochemicals derived from medicinal plants serve as primary compounds in the process of drug discovery and design. Certain chemicals are synthetically produced to mimic the natural properties of plants, whereas other compounds are obtained directly from plant extracts.[5,6]
One member of the Rosaceae family with medical uses is *Prunus avium*. For thousands of years, mankind have survived on the fruits of wild cherries. The material is rich in vitamins and minerals and contains a multitude of phytochemicals that are closely linked to its antioxidant capacity.[7,8] Because of its high concentration of antioxidant compounds, the *Prunus avium* fruit is recognized for its medicinal qualities, which include anticancer, nutritional, antioxidant, and anti-inflammatory benefits.

Figure 1. *Prunus avium*

Cedrus deodara belongs to the Pinaceae family of plants. The complete plant has therapeutic value. The plant's many parts, including its wood, leaves, oil, and gum, have different nutritional and therapeutic uses.[9,10]

Figure 2. *Cedrus deodara*

2. METHODS

2.1 Preparation of extracts

The leaves of *Prunus avium* and *Cedrus deodara* were shade-dried separately. Powder is ground into a fine consistency using a grinder. Ethanol and distilled water were used to extract the leaf powder in the Soxhlet device. The extract was then hot-filtered following that. Via the distillation process, solvents are eliminated. This entirely removes the solvent by lowering the pressure.

2.2 Experimental Animals

Albino from Switzerland the experiments will be conducted with mice weighing 20–50 grammes. We'll get the mice from a reliable supplier. The animals will be housed a 12-hour light and dark cycle, unlimited access to pellet food and water, and plastic enclosures maintained at 22±3°C. Three times a week, thorough clearing and getting rid of excrement from cages will ensure optimal hygiene. Prior to the trial, the mice spent a week becoming used to the lab setting. Food will be withheld for eighteen hours prior to the start of each trial. Water was accessible, though, with the exception of entry pools, when food and water are removed. International criteria for the upkeep and use of experimental animals will be followed in the handling and care of the animals.

2.3 Grouping and Dosing:

Only the castor oil-induced diarrhea animal model—which is split into seven groups, each with six animals—will be utilized in the main detection of the antidiarrheal activity of plant extracts. Four out of the five animal models will need to undergo animal experiments in order to discover antidiarrheal activity after the most efficient extract of all three plants has been identified.

Each animal model is further divided into 5 groups:
- Negative control Group
- Positive Control Group
- Test Drug Extract 1 group
- Test Drug Extract 2 group
- Test Drug Extract 3 groups and each group will be having 6 animals.

The positive control group's models will all receive 0.5ml of castor oil pomade as a diarrhoea inducer and 3mg/kg of loperamide as a normal medication. The negative control group will receive 10mg/kg/p.o. of purified water. Each test drug will have three dosages chosen based on the results of an acute toxicity test. The intermediate dose is determined by taking the dose used in the acute toxicity test (1/10th), and the highest and lowest doses are determined by taking the 2X dose and half of the middle dose.

2.4 Castor oil induced Diarrhoea:

To induce diarrhoea in the mouse model, castor oil was employed as part of the experimental protocol. Swiss albino mice, both male and female, subjected to an after an eighteen-hour fast, were divided into five groups, with five animals in each group. After the administration of appropriate doses of loperamide and extract to each animal, as delineated in the grouping and dosing section, every mouse received 0.5 mL of castor oil. The mice were continuously observed for a four-hour duration, during which the researchers recorded the frequency of defecation, the weight of each mouse's excrement, and the start of the diarrhea including both wet and total weight.

The following equations were employed to be calculated:

Percent of wet feces Inhibition:

$$\frac{\text{Average no. of wet feces of control} - \text{Average No. of wet feces of Drug Treated Group}}{\text{Average no. of wet feces of control}} \times 100$$

Percentage of Wet Fecal Out Put:

$$\frac{\text{Average weight of wet feces of control} - \text{Average weight of wet feces of Drug Treated Group}}{\text{Average weight wet feces of control}} \times 100$$

Percentage of total Fecal Out Put:

$$\frac{\text{Average weight of total feces of control} - \text{Average weight of total feces of Drug Treated Group}}{\textit{Average weight of total feces of control}} \times 100$$

2.5 Castor Oil Induced Enterpooling:

A total of thirty mice, including both males and females, will be separated into five groups at random, each comprising six mice. These mice will undergo an eighteen-hour fasting period, during which they will be deprived of both food and drink. As mentioned in the grouping and dose section, each animal will be given 0.5 mL of castor oil orally one hour after the extract and loperamide are given. After administering castor oil for one hour, all mice will be euthanized using cervical dislocation. Subsequent to the ligation of the pyloric end and ileocecal junction, Every mouse will have surgery to open its belly, and the small intestine will be meticulously removed and weighed. A graduated tube containing the compressed intestinal contents will have its volume measured. The disparity between the weight of the small intestine with contents and the empty small intestine will be calculated upon reweighing the gut.

These parameters will provide valuable insights into the impact of the administered substances on intestinal weight and content.

Percent of Inhibition using Intestinal Weight with content:

$$\frac{\text{Mean Weight of intestine with content for control- Mean Weight of intestine with content for Drug Treated}}{\text{Mean Weight of intestine with content for control}} \times 100$$

Percent of Inhibition using Volume of Intestinal Content:

$$\frac{\text{Mean Volume of Intestinal Content for control- Mean Volume of Intestinal Content for drug treated}}{\text{Mean Weight of intestine with content for control}} \times 100$$

2.6 Castor Oil Induced Gastrointestinal Motility

After an eighteen-hour fasting period, Five groups of thirty mice were randomly assigned, each comprising five animals. Subsequently, the mice underwent treatment following the guidelines provided in the animal grouping and dosage section. An hour following the administration of castor oil, each mouse received 1 mL of

a marker solution, consisting of a 5 percent suspension of distilled water with activated charcoal. After another one-hour period, during which the activated charcoal was allowed to traverse the small intestines, all mice were euthanized. The small intestines, extending from the stomach to the colon, were extracted and positioned on a sterile surface. A thorough inspection was conducted to measure the distance from the pylorus to the cecum. The proportion of this total length covered by the charcoal meal was then determined, and the peristaltic indexa percentage was named after it. This methodology facilitated the assessment of the impact of the administered substances on peristaltic activity in the small intestines of the mice. The inhibition % was subsequently calculated using a method.

Peristalsis Index:

Distance Travelled by Charcoal × 100 Length of Intestine

Percent Inhibition:

Peristalsis Index of Control Group - Peristalsis Index of Drug Treated Group Peristalsis Index of Control Group

2.7 In vivo Anti-Diarrheal Index

The antidiarrheal index (ADI) in vivo was determined for both the group treated with the extract and the group under positive control. This was done by utilizing data obtained from the aforementioned tests and applying the following formula:

$$ADI = \sqrt[3]{D\ freq \times G\ meq \times P\ freq}$$

2.8 Tablet Formulation

Sl. No.	Ingredients (mg)	F1	F2	F3	F4	F5	F6
1	Plant extract	600	600	600	600	600	600
2	Lactose	20	30	20	20	30	20
3	Starch	20	10	20	-	-	-
4	Gum Acacia	-	-	-	20	10	20
5	Magnesium stearate	6	6	6	6	6	6
6	Talc	4	4	4	4	4	4
Total weight 650mg		650	650	650	650	650	650

Table 1. Formulation of tablet

2.9 Evaluation of herbal tablet

2.9.1 Uniformity of Weight

Twenty tablets from each formulation that were chosen at random were weighed separately. The weights of the individual tablets were then contrasted with the average weight.

2.9.2 General appearance

We noted and examined the colour, smell, and texture of the tablet in addition to its general look.

2.9.3 Hardness test

Using the Monsanto hardness tester, the hardness of twenty randomly chosen tablets from each formulation was determined. The tablets must have a certain degree of firmness or hardness in all procedures and be able to endure mechanical shocks when being handled.

2.9.4 Percentage friability test

The Roche Friabilator was used to evaluate the friability of tablets. Using the friability instrument, the weight loss percentage of twenty tablets that were randomly chosen from each batch were determined. After four minutes of spinning at 25 rpm and the removal of any tablet dust, the percentage of weight loss was calculated.

2.9.5 Disintegration test

Tablet disintegration time was measured using a digital microprocessor-based disintegration test instrument. One tablet and one disc were placed inside each tube, and the entire assembly was suspended in a water-filled 1000 mL beaker. The water level was adjusted so that the wire mesh was at least 25 mm below the top of the water at its highest point and at least 25 mm above the bottom of the beaker at its lowest point. The device was maintained and operated at 37±2°C, and the duration needed for every tablet to break down and enter the wire mesh was noted.

2.9.6 In vitro dissolution studies

The manufactured tablets underwent investigations on their in vitro disintegration at 37±0.5ºC using USP equipment II. The experiments were conducted at a speed of 50 rpm using 900 mL of 0.1N HCl solution as the dissolution media. At preset intervals of 10, 20, 30, 45, 60, and 75 minutes, samples were removed, and 5 mL of the original medium were replaced with a corresponding amount of fresh medium kept at 37±0.5ºC. These samples were passed via a 0.45 µm Millipore membrane filter and then submitted to spectrophotometric examination at 315 nm. The average outcomes of three sets of dissolving tests are presented.

2.10 Stability Studies

Temperature, light, air, humidity, and other elements of the storage environment can all have an impact on a medication dosage form's stability criterion. Under long-term testing circumstances (25±2ºC/60 percent RH+5ºC) and for six months under accelerated temperature conditions (40ºC/75 percent RH+5ºC), each formulation was subjected to stability testing.

3. RESULTS

3.1 Determination of Antidiarrheal Activity of *P. avium*

3.1.1 Effects of P. avium Leaf Extract on Castor Oil–Induced Diarrheal Model

Mice treated with extracts exhibited a significant antidiarrheal effect in the castor oil-induced diarrhoea test. Methanol extract notably reduced the frequency of defecation ($P<0.05$) compared to untreated control rats. Furthermore, the extracts reduced the overall quantity of wet feces compared to mice treated with castor oil. These results demonstrate the potential of the extracts in mitigating diarrhoea symptoms in experimental models.

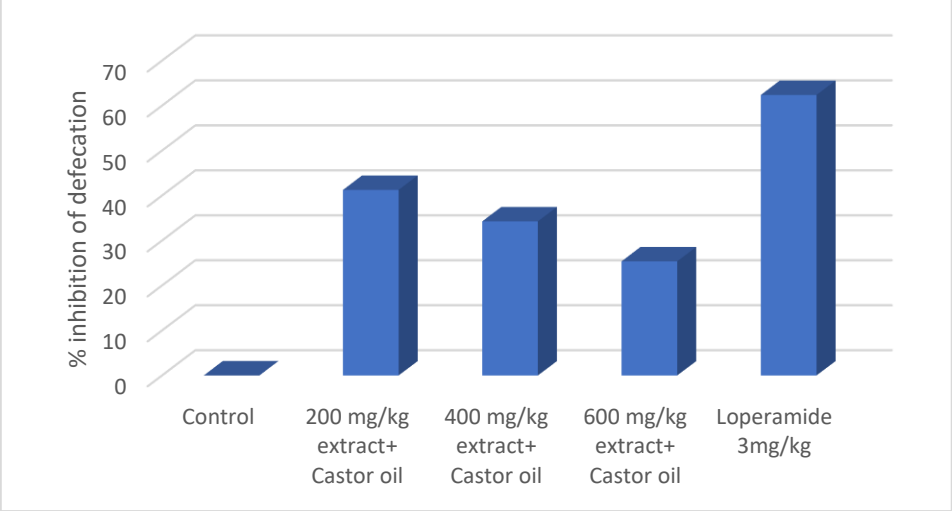

Figure 3. % inhibition of defecation

3.1.2 Effects of *P. avium* Leaf Extract on castor oil induced enterpooling in mice

In the castor oil-induced enteropooling test, *P. avium* extracts demonstrated a notable impact on mice, resulting in a 29.75% decrease in intestinal volume. Statistical significance ($P<0.05$) was observed. While the extract's effect was less potent than the conventional medication loperamide (3 mg/kg), which significantly prevented a 70.19% increase in intestinal fluid buildup ($P<0.01$), these findings underscore the potential of *Prunus avium* extracts in modulating intestinal fluid dynamics and warrant further investigation.

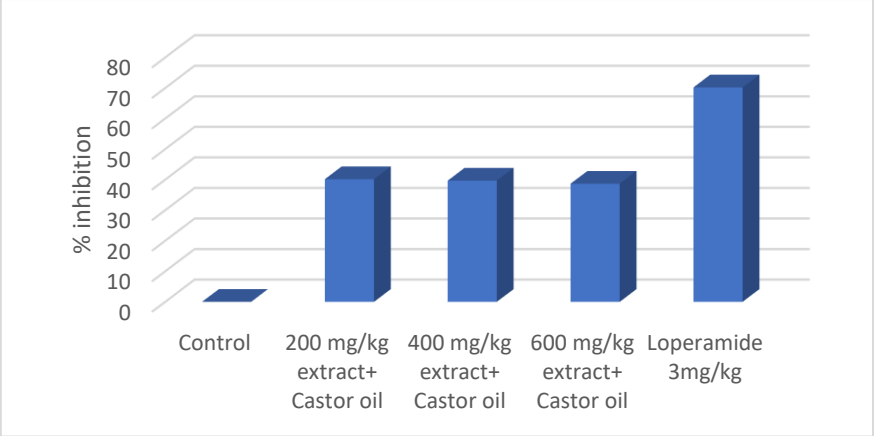

Figure 4. % Inhibition in mice

3.1.3 Effects of *P. avium* Leaf Extract on Gastrointestinal motility test

In contrast to the group under control, mice treated with the extracts exhibited a significant ($P<0.01$) reduction in gastrointestinal distance travelled after ingesting charcoal. Additionally, the passage of charcoal meal through the digestive tract was significantly reduced with loperamide (3 mg/kg) treatment. These results suggest the potential of the extracts in modulating gastrointestinal motility.

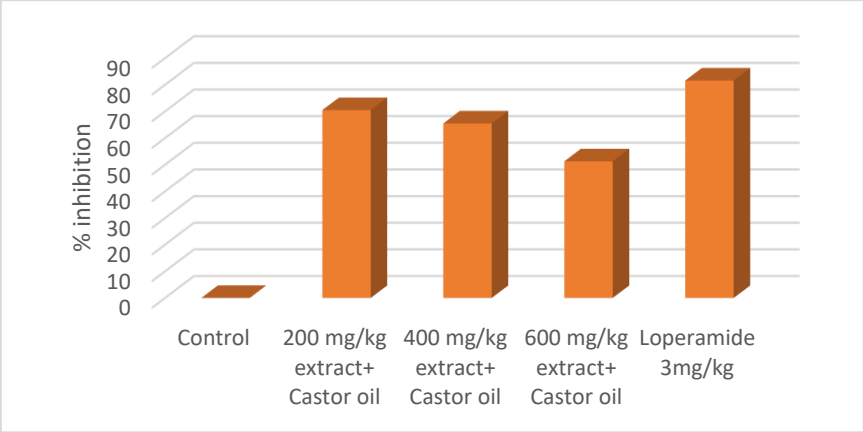

Figure 5. % inhibition of Gastrointestinal Fluid Accumulation in Mice

3.1.4 In Vivo Antidiarrheal Index

The cumulative impact of the plant-based extraction the regularity of bowel movements, the beginning of loose stool, and the accumulation of intestinal fluid is quantified by the ADI. Plant-based extracts had ADI values of 62.34, 92.64, and 97.65 respectively. According to these findings, the plant extract exhibited a dose-dependent antidiarrheal index, reaching its peak at 600 mg/kg.

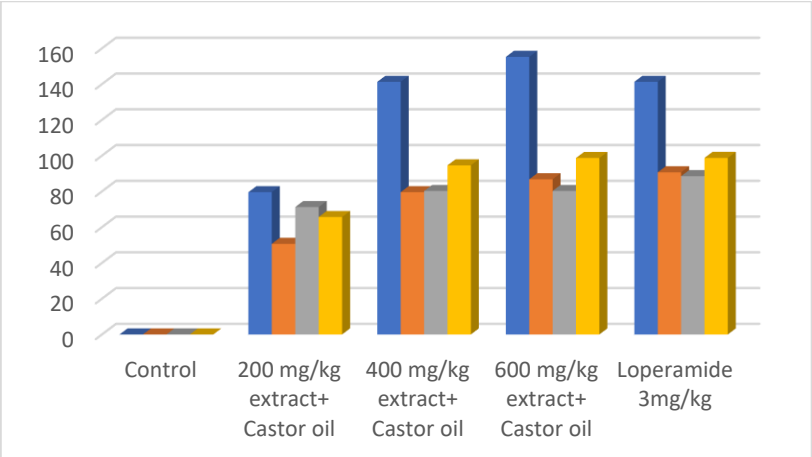

Figure 6. Percentage of Antidiarrheal Indices

3.1.5 Evaluation of herbal tablets

Parameters	F1	F2	F3	F4	F5	F6
Uniformity of weight	1.03±0.51	1.45±0.68	1.49±0.85	1.79±0.77	1.50±0.75	1.60±0.62
Colour	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Hardness (kg/cm2)	5.56±0.01	4.20±0.51	3.01±0.10	2.26±0.38	4.20±0.01	4.20±0.18
Friability (%)	0.68±0.02	0.71±0.02	0.75±0.04	0.68±0.02	0.72±0.02	0.75±0.01
Disintegration time (minutes)	14.09±0.80	11.51±0.41	13.09±0.65	13.54±0.75	12.00±0.52	13.44±0.66

Table 2. Evaluation parameters of tablets of *P. avium*

3.1.6 In-vitro dissolution study

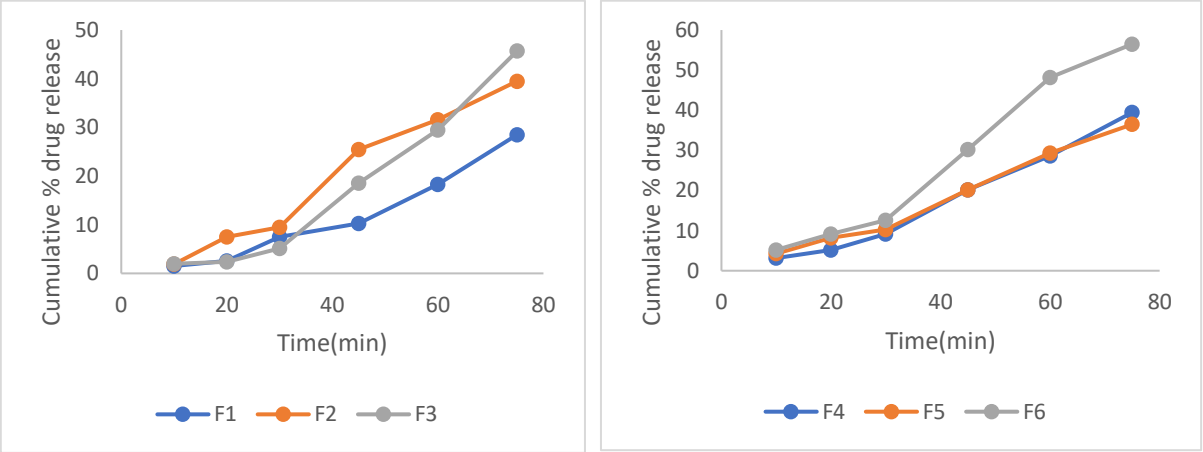

Figure 7. Dissolution profile of tablets (F1 to F6)

3.1.7 Stability studies

After three months, no formulation shown any significant differences in any of the parameters, leading to the selection of the F2 formulation for stability studies.

Parameters	Initial	Long term				Accelerated	
		3month	6month	9month	12month	3month	6month
Uniformity of weight	1.45±0.68	1.41±0.71	1.44±0.67	1.42±0.67	1.39±0.70	1.63±0.97	1.39±1.14
Color	Dark brown	No change	No change	No change	No change	No change	Brown
Odor	Characteristic	No change	No change	No change	No change	No change	No change
Texture	Smooth	No change	No change	No change	No change	No change	No change
Hardness (kg/cm2)	6.98±0.142	6.96±0.153	6.955±0.147	6.955±0.135	6.955±0.147	6.94±0.11	6.92±0.14
Friability (%)	0.73±0.02	0.72±0.02	0.73±0.01	0.74±0.01	0.74±0.01	0.75±0.02	0.77±0.002
Disintegration time (minutes)	12.19±0.51	12.75±0.52	12.74±0.41	12.79±0.45	12.76±0.65	11.28±0.76	10.59±0.62

Table 3. Stability studies for *P. avium* tablets

3.2 Determination of Antidiarrheal Activity of *Cedrus deodara*

3.2.1 Effects of *Cedrus deodara* Leaf Extract on Castor Oil–Induced Diarrheal Model

Mice treated with extracts exhibited a significant antidiarrheal effect in the castor oil-induced diarrhea test. Methanol extract significantly reduced the frequency of defecation ($P<0.05$) compared to untreated control rats. Furthermore, compared to mice treated with castor oil alone, the extracts significantly decreased the overall quantity of wet feces. These findings underscore the potential of the extracts in mitigating diarrhea symptoms in experimental models.

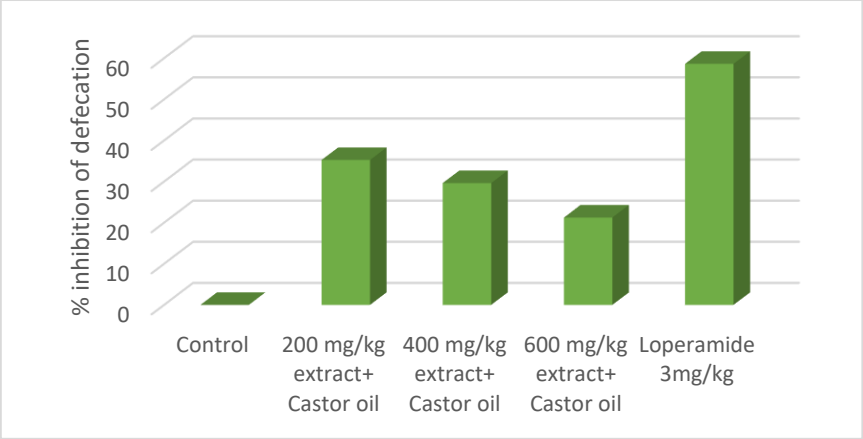

Figure 8.% inhibition of defecation

3.2.2 Effects of *Cedrus deodara* Leaf Extract on castor oil induced enterpooling in mice

In the castor oil-induced enteropooling test, *Cedrus deodara* extracts exhibited a noticeable impact on mice, resulting in a 28.67% decrease in intestinal volume. The observed effect, though less potent than loperamide (3 mg/kg), was statistically significant. Loperamide significantly reduced intestinal fluid buildup by 68.49%. These findings suggest that *Cedrus deodara* extracts may possess potential anti-diarrheal properties, warranting further investigation for therapeutic applications.

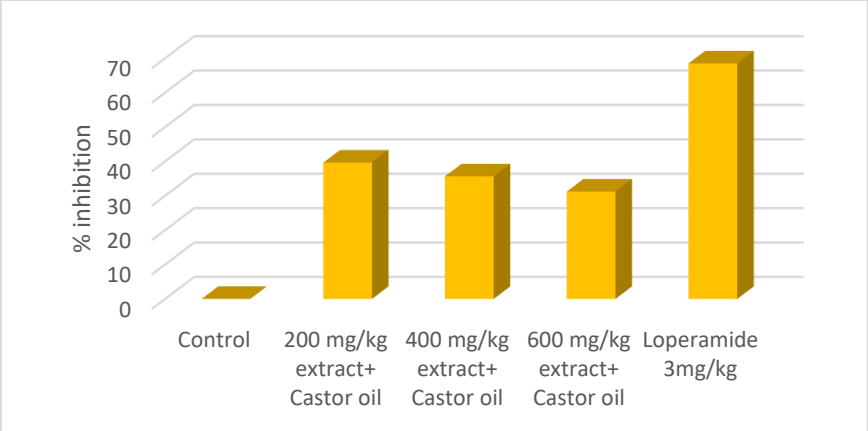

Figure 9. % inhibition on castor oil induced enterpooling in mice

3.2.3 Effects of *Cedrus deodara* Leaf Extract on Gastrointestinal motility test

In contrast to the group under control, the extracts significantly reduced the gastrointestinal distance travelled by mice after ingesting charcoal. Loperamide (3 mg/kg) also significantly decreased the passage of meal made of charcoal via the digestive system, showing a notable reduction of 41.06%.

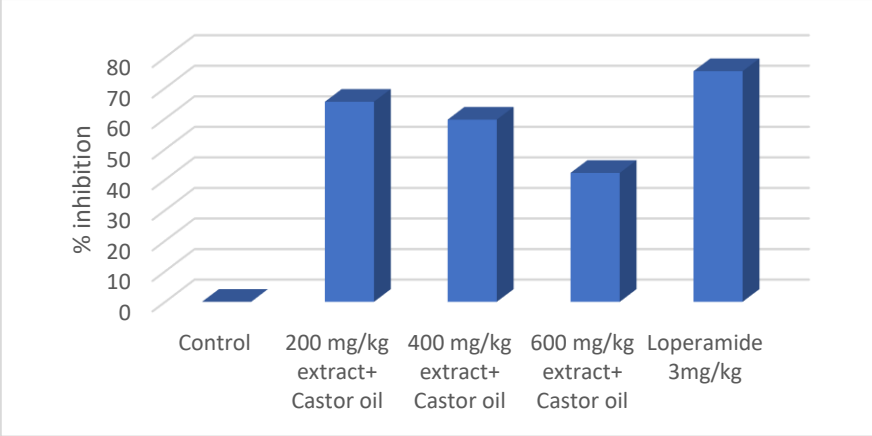

Figure 10. % inhibition of Gastrointestinal Fluid Accumulation in Mice

3.3.4 In Vivo Antidiarrheal Index

The Antidiarrheal Index (ADI), incorporating effects on bowel movement frequency, onset of diarrhoea, and intestinal fluid buildup, exhibited dose-dependent trends. At dosages of plant extract ADI values were 62.34, 92.64, and 97.65, respectively. The findings suggest a dose-dependent antidiarrheal effect, reaching its peak at 600 mg/kg, emphasizing the potential of the plant extract in mitigating diarrhoea symptoms in a concentration-dependent manner.

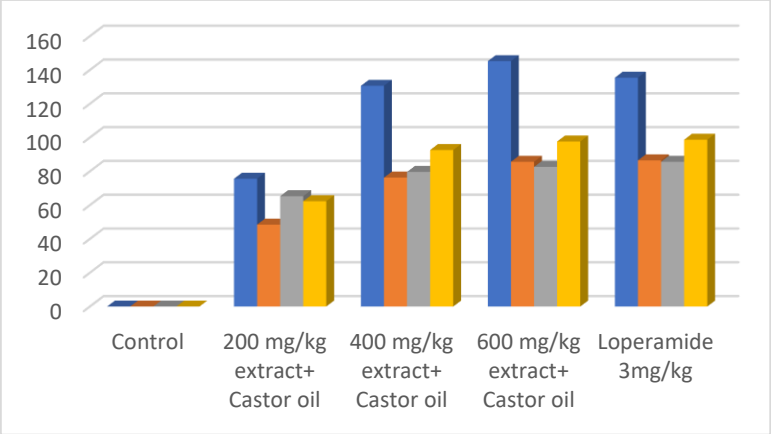

Figure 11. Percentage of Antidiarrheal Indices

3.3.5 Evaluation of herbal tablets

Parameters	F1	F2	F3	F4	F5	F6
Uniformity of weight	1.03±0.51	1.45±0.68	1.49±0.85	1.79±0.77	1.50±0.75	1.60±0.62
Colour	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Hardness (kg/cm2)	7.05±0.123	6.98±0.142	6.99±0.159	7.02±0.148	6.94±0.154	7.01±0.146
Friability (%)	0.68±0.02	0.71±0.02	0.75±0.04	0.68±0.02	0.72±0.02	0.75±0.01
Disintegration time (minutes)	14.09±0.80	11.51±0.41	13.09±0.65	13.54±0.75	12.00±0.52	13.44±0.66

Table 4. Evaluation parameters of tablets of *Cedrus deodara*

3.3.6 In-vitro dissolution study

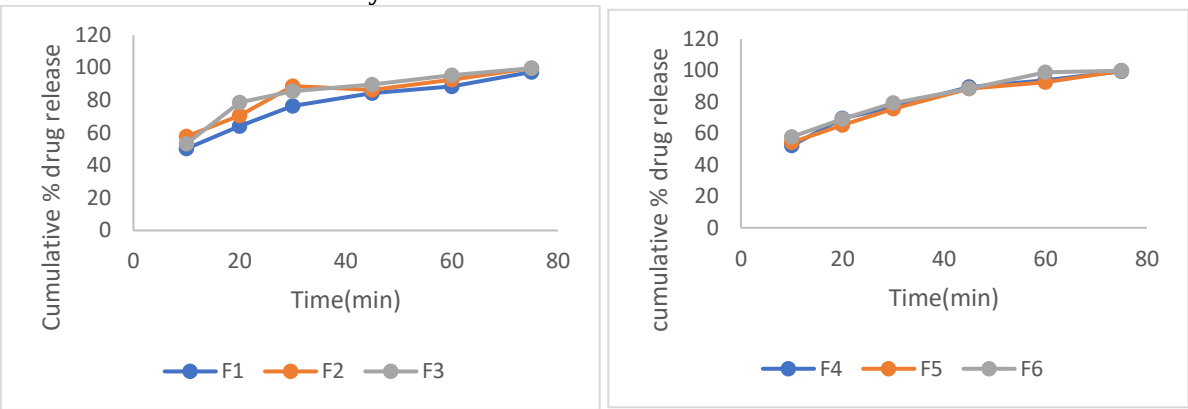

Figure 12. Dissolution profile of tablets (F1 to F6)

3.3.7 Stability studies

Parameters	Initial	Long term				Accelerated	
		3month	6month	9month	12month	3month	6month
Uniformity of weight	1.45±0.68	1.41±0.71	1.44±0.67	1.42±0.67	1.39±0.70	1.63±0.97	1.39±1.14
Color	Dark brown	No change	No change	No change	No change	No change	Brown
Odor	Characteristic	No change	No change	No change	No change	No change	No change
Texture	Smooth	No change	No change	No change	No change	No change	No change
Hardness (kg/cm2)	6.98±0.142	6.96±0.153	6.955±0.147	6.955±0.135	6.955±0.147	6.94±0.11	6.92±0.14
Friability (%)	0.71±0.02	0.72±0.02	0.73±0.01	0.74±0.01	0.74±0.01	0.75±0.02	0.77±0.002
Disintegration time (minutes)	11.51±0.41	11.46±0.45	11.40±0.31	11.38±0.39	11.31±0.43	10.51±0.73	09.48±0.45

Table 5. Stability studies for *Cedrus deodara* tablets

4. CONCLUSION

The antidiarrheal properties of the *Cedrus deodara* and *P. avium* leaf extract were evaluated using Swiss albino mice as animal models. The plant extract significantly slowed down the onset of diarrhoea and decreased its frequency of wet feces, and demonstrated strong antisecretory effects at all experimentally examined doses. This study focuses on the formulation and evaluation of tablets derived from the leaf extracts of *Cedrus deodara* and *P. avium*. Herbal products often feature combinations of multiple herbs for synergistic or complementary effects, or they may consist of a single herb. The investigation aims to understand the potential benefits and properties of these specific plant extracts when formulated into tablet form, contributing valuable insights into herbal formulations for various applications.

5. REFERENCES

1. Chaudhary, Amrendra Kumar, Shamim Ahmad, and Avijit Mazumder. "Cedrus deodara (Roxb.) Loud.: a review on its ethnobotany, phytochemical and pharmacologicalprofile." *Pharmacognosy Journal* 3.23 (2011): 12-17.
2. Sharma, Parth, Surajpal Verma, and Plakshi Misri. "Global need for novel herbal drug formulations." *Int. J. Phcog Phytochem. Res* 8.9 (2016): 1535-1544.
3. Beri, Chiman, and Isha Sacher. "Development of fast disintegration tablets as oral drug delivery system-A review." *Indian Journal of Pharmaceutical and Biological Research* 1.03 (2013): 80-99.
4. Pahwa, Rakesh, et al. "Orally disintegrating tablets-Friendly to pediatrics and geriatrics." *Archives of*

applied science research 2.2 (2010): 35-48.
5. Sharma, Deepak. "Fast disintegrating tablets: a review on an emerging trend in novel oral drug delivery technology and new market opportunities." *Journal of drug delivery and therapeutics* 2.3 (2012).
6. Guidance for Industry 1: Orally disintegrating tablets. U. S. Food and Drug Administration. www.fda.gov/cder/Guidance/5909dft.htm#_Toc462 221103
7. Guerin, E., et al. "Rheological characterization of pharmaceutical powders using tap testing, shear cell and mercury porosimeter." *International journal of pharmaceutics* 189.1 (1999): 91-103.
8. Kiran, Dhakane, et al. "Fast dissolving tablet: A Future prospective." *Journal of Pharmacy Research* 4.11 (2011): 4176-4180.
9. Electronic edition, Rockvelle, MD. United State Pharmacopoeial convention Inc; 2007.
10. Gupta, A., et al. "Recent trends of fast dissolving tablet-an overview of formulation technology." *International Journal of Pharmaceutical & Biological Archives* 1.1 (2010): 1-10.