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Evaluation Of Antidiarrhoeal Activity Of Ethanolic Leaf Extract Of *Woodfordia Fruticose And Rubus Ellipticus* In Swiss Albino Mice

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Abstract

One of the main preventable causes of death in developing nations is diarrhoea. According to reports, the leaves of *Woodfordia fruticose* and *Rubus ellipticus* have traditionally been employed as an antidiarrheal remedy. The current study sought to assess the pharmacological activity of an ethanolic extract of *Woodfordia fruticose* and *Rubus ellipticus* leaf in the treatment of diarrhoea. The extract was utilized to treat castor oil-induced diarrhea in mice. The extracts were given to mice orally at dosages of 100, 200, and 400 mg/kg bodyweight, with loperamide serving as a typical diarrhea treatment. The results demonstrated % inhibiting diarrhoea with values of no detectable, 43.68%, 82.98%, and 71.42% at 100, 200, and 400 mg/kg bodyweight levels of dose of the extract and loperamide, respectively, as compared to the negative control. At a dose of 400 mg/kg bodyweight, the extract has a stronger antidiarrheal effect than loperamide. In conclusion, an ethanolic extract of *Woodfordia fruticose* and *Rubus ellipticus* leaves shows anti-diarrheal activity, supporting use this plant as an antidiarrheal in traditional medicine.

Keyword: - Woodfordia fruticose, Rubus ellipticus, diarrhoea

1. INTRODUCTION

The alteration in stool consistency, heightened frequency of defecation, and an escalation in fecal volume or liquidity collectively signify the manifestation of diarrhea. It is not feasible to measure the amount of fluid contained in stools; instead, it is more appropriate to evaluate the frequency of stools for diagnostic purposes.[1,2] The symptoms of diarrhea encompass an augmented coupled with an intensified secretion, along with a diminished capacity for fluid absorption.[3,4] The unusually frequent and watery stools that come from the fast transit of feces through the colon are found in those who have diarrhea.[5,6] When a patient has this condition, they typically complain of stomach cramps and widespread weakness. Additionally, their ability to absorb water, nutritional components, and electrolytes is reduced. Stools that are normal for an adult who is healthy weigh between 100 and 200 grams and include sixty percent water. diarrhea can be caused by even a slight change in weight (excretion of more than 20 grams) and the percentage of water (60–90%) as the primary component.[7,8]

During the early 1980s, diarrheal diseases stood as the predominant cause of mortality among children, contributing to an estimated four to six million deaths globally each year. Despite the extensive adoption of oral rehydration treatments (ORT) and an enhanced comprehension of diarrhea's root causes, an alarming two to five million children continue to succumb to these maladies annually. Notably, the brunt of these fatalities is borne by underdeveloped nations.[9,10]

The indigenous species *Woodfordia fruticosa* is found across India, reaching elevations of up to 1500 meters. It is additionally present in other nations. It is situated in northeastern India in the Tenga and Salari regions, which extend to Nafra. The main place to find it is in Mizoram, particularly in the Kawlkuth regions. Furthermore, it can be found in a tiny area of West Bengal's northern region. It can also be found in the Gangetic plains and the Shivalik mountain range in Himachal Pradesh. [10,11]

Figure 1. *Woodfordia fruticose* leafs

R. ellipticus is a Rosaceae family member that is commonly found on the edges of woods. It's used to make jam, squash, ice cream, and yoghurt. It has a lot of potential in the agro-processing industries and provides essential nutrients for human nutrition. This small fruit is found in the Himalayas at heights between 700 and 2000 meters above sea level. It is widely distributed throughout the world. It reaches the Chinese province of Yunnan and is also found in Bhutan, West Sikkim, the Khasi highlands, and Burma. [12,13]

Figure 2. *Rubus ellipticus* leafs

2. METHODS

2.1 Preparation of extracts

The leaves of *Rubus ellipticus and Woodfordia fruticosa* were shade-dried independently. Powder is ground into a fine consistency using a grinder. Ethanol and distilled water were used in the Soxhlet equipment to extract the powdered roots. 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

Mice weighing between twenty and fifty grams will be used in this study. They will be purchased from a reliable source in Switzerland. The mice will live in plastic cages with unlimited access to water and pellet food. The enclosures will have a 12-hour light and dark cycle and be kept at a temperature of 22±3 ºC. Three times a week, cages will be completely cleaned and waste removed to maintain hygienic conditions. The mice will

spend a week in lab settings prior to the start of the experiments. Before every trial, food will be denied for eighteen hours. Only the official entry pools—where food and water are provided—will have access to water. Strict adherence to international rules on the use and care of experimental animals shall be observed with regard to handling and maintenance.

2.3 Grouping and Dosing of Animals

A random assignment produced five groups of three mice each, in addition to two control groups consisting of five mice each. Each cohort received the appropriate treatments via oral gavage. The first group received 10 milliliters of DMSO per kilogram as the negative control, while the fifth group received 3 milligrams of loperamide per kilogram as the positive control. Doses of 100, 200, and 400 mg/kg of the extract were administered to the second, third, and fourth groups, respectively. The acute toxicity test determined the following dosages for the 80% ethanol: 100, 200, and 400 mg/kg. To clarify, the minimum and maximum doses were determined by ingesting half and twice the middle dose, respectively.

On the day of the experiments, the animals were administered a freshly prepared extract solution that had been reconstituted with the appropriate quantities of DMSO. The positive control, loperamide, was administered to the animals in a comparable fashion subsequent to its reconstitution in DMSO. Following the determination of the mg/kg required for each animal, the equivalent volume of the reconstituted solution containing the required mg of extract or loperamide was computed for each animal. This facilitated the delivery of the corresponding quantities to the groups that were treated with extract and loperamide.

2.4 Determination of Antidiarrheal Activity

2.4.1 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 diarrhoea including both wet and total weight.

The percentages of diarrhoea inhibition and fecal output weight were computed using the following formulas:

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:

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

2.4.2 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. One hour after the castor oil administration, each mouse received 1 mL of a marker solution, consisting of a 5 percent suspension of activated charcoal in distilled water. 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 pylorus to the cecum, were extracted and placed 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

2.4.3 Castor Oil Induced Enterpooling

A total of thirty mice, including both males and females, will be divided 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 stated 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, permitting the percentage of inhibition to be calculated as a gauge of the treatments efficacy.

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:

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

2.5 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:

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

3. RESULTS

3.1 Determination of Antidiarrheal Activity of *Woodfordia fruticose*

3.1.1 Effects of *Woodfordia fruticose* Leaf Extract on Castor Oil–Induced Diarrheal Model

At a dose of 400 mg/kg percentage of defecation inhibition (88 percent) is greater, it is quite similar to standard drug.

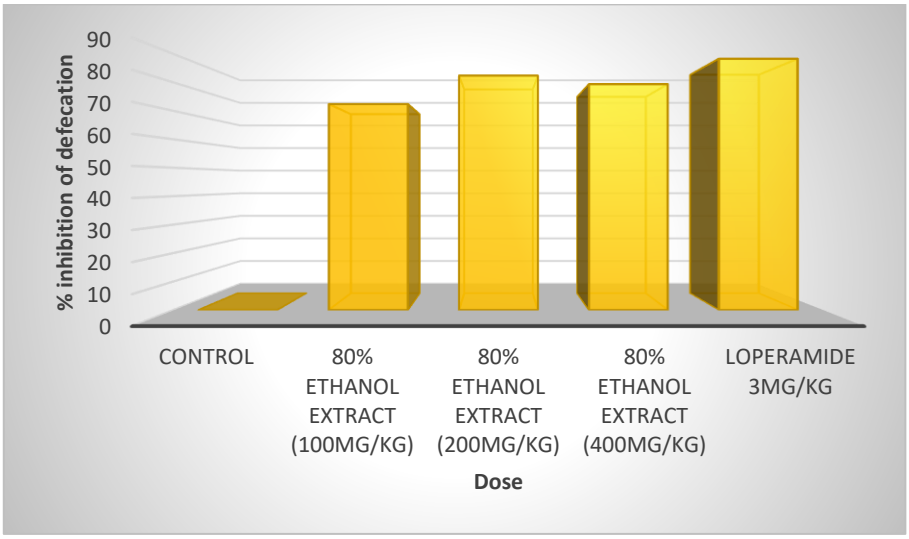

Figure 3. Percentage inhibition of *Woodfordia fruticose*

3.1.2 Effects of *Woodfordia fruticose* Leaf Extract on Castor Oil–Induced Gastrointestinal Transit

In the absence of positive treatment influence, the peristaltic indicator of the charcoal repast within the control cohort reached a value of 75.65±4.25. The standard medication revealed a marked decrease charcoal meal, accomplishing the highest percentage of hindrance when juxtaposed with the control cohort.

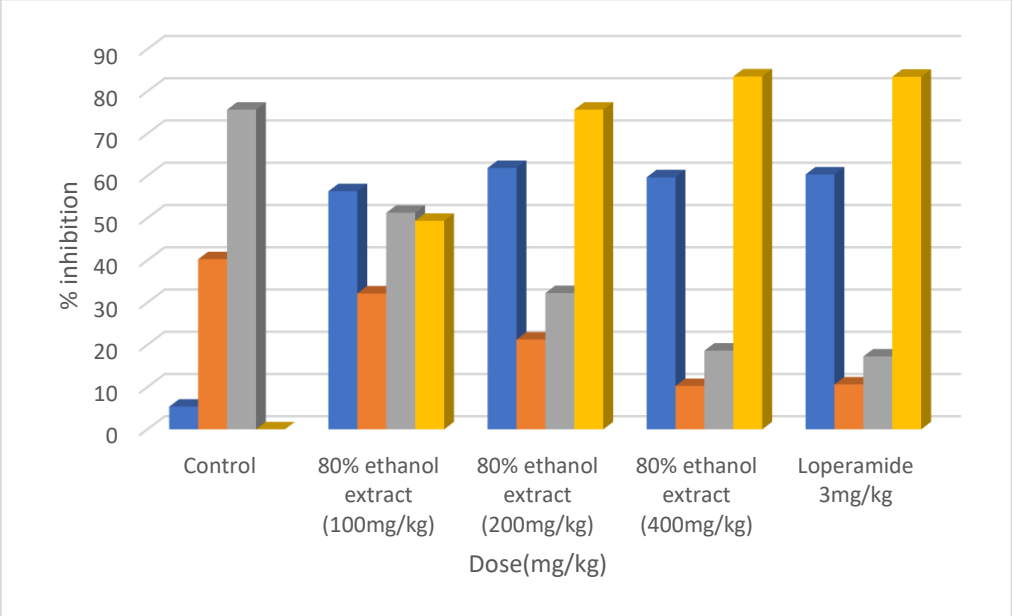

Figure 4. *Woodfordia fruticose* on Gastrointestinal Transit in Mice

3.1.3 Effects of *Woodfordia fruticose* Leaf Extract on Gastrointestinal Fluid Accumulation

The negative control group exhibited an intestinal contents volume of 0.87±0.18and a weight of 1.20±0.09, respectively. The accumulation of gastrointestinal fluid induced by castor oil was significantly reduced by all dosages of the plant extract. Consequently, the intestinal contents volume for the groups treated with extract at different doses 0.36 ± 0.05, 0.34 ± 0.04, and 0.30 ± 0.03. Additionally, the weight of intestinal contents at 100 mg/kg (0.62±0.09), 200 mg/kg (0.61±0.21), and 400 mg/kg (0.63±0.20) was significantly reduced by the plant extract compared to the control group.

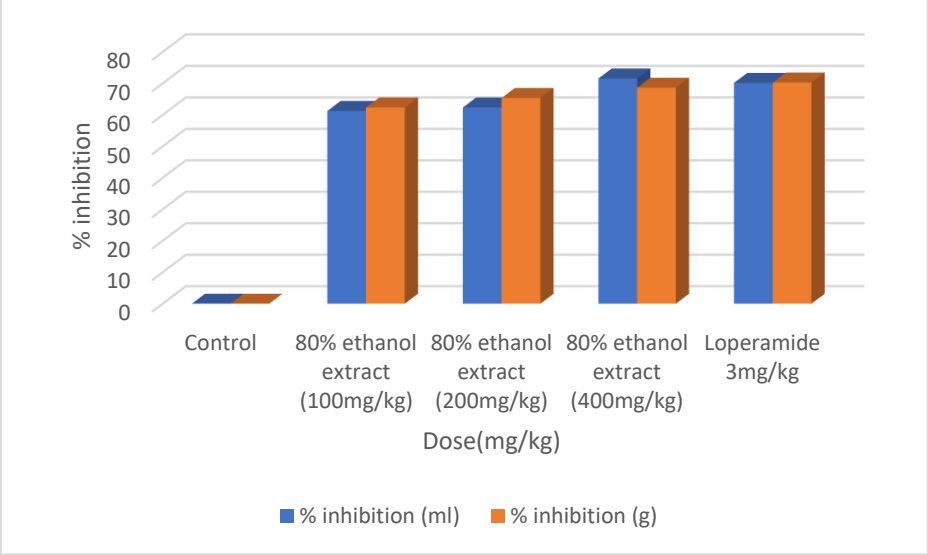

Figure 5. % inhibition of volume and weight of intestinal contents of *Woodfordia fruticose*

3.1.4 In Vivo Antidiarrheal Index

Plant extracts at different dosages shows ADI values of 63.56, 89.64, and 96.72, respectively. According to these findings, the plant extract demonstrated a dose-dependent antidiarrheal index, reaching its peak at 400 mg/kg.

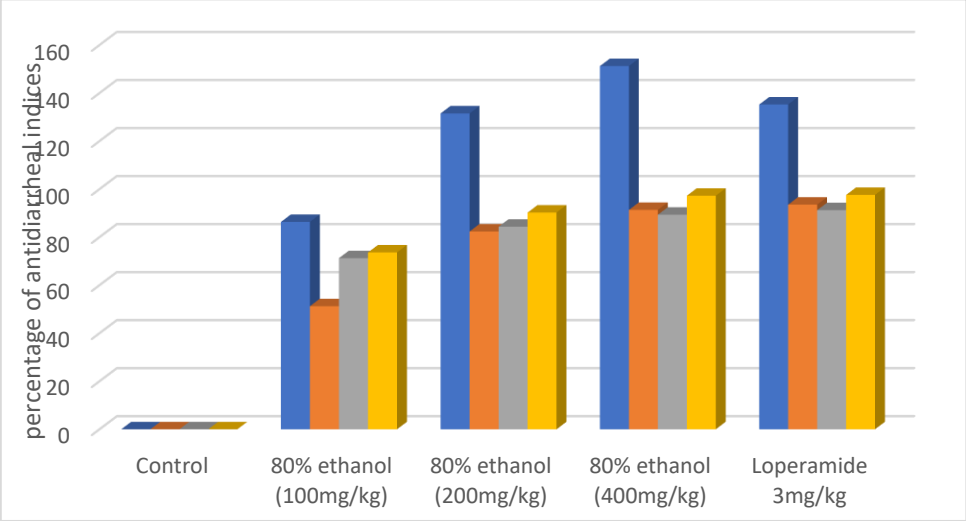

Figure 6. Antidiarrheal Index of *Woodfordia fruticose*

3.2 Anti-diarrheal activity of *Rubus Ellipticus*

3.2.1 Evaluation of the Antidiarrheal Effects of Rubus Ellipticus Leaf Extract on Castor Oil–Induced Diarrheal Model

The study investigated the antidiarrheal potential of the 80% ethanol extract derived from *Rubus Ellipticus* leaves in a castor oil-induced diarrhoea model. The findings revealed a noteworthy delay in the onset of diarrhoea at all administered test doses. At 400 mg/kg dose the inhibition defecation level reaches to 78.57%. It was discovered that the observed efficacy was equivalent to that of the prescription drug loperamide. The study underscores the promising antidiarrheal effects of *Rubus Ellipticus* leaf extract, supporting its potential therapeutic utility in managing diarrheal.

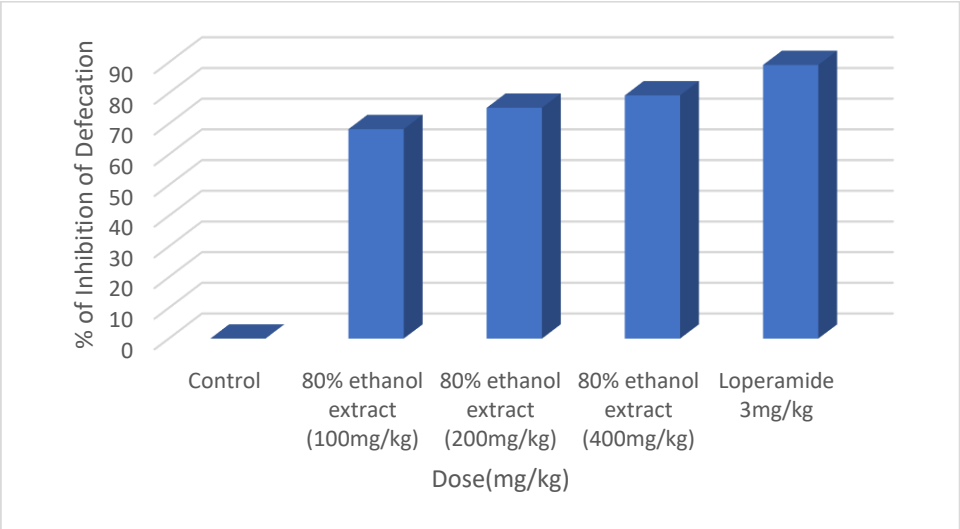

Figure 7. % inhibition of defecation

3.2.2 Effects of *Rubus Ellipticus* Leaf Extract on Castor Oil–Induced Gastrointestinal Transit

The peristaltic index of the charcoal meal was measured at in the negative control group at 78.25±5.36. Administration of the plant extract significantly reduced the charcoal meal's travel distance at doses of 200 mg/kg (13.26±5.36) and 400 mg/kg (9.6±4.36). result show a significant reduction in marker as compare to normal group.

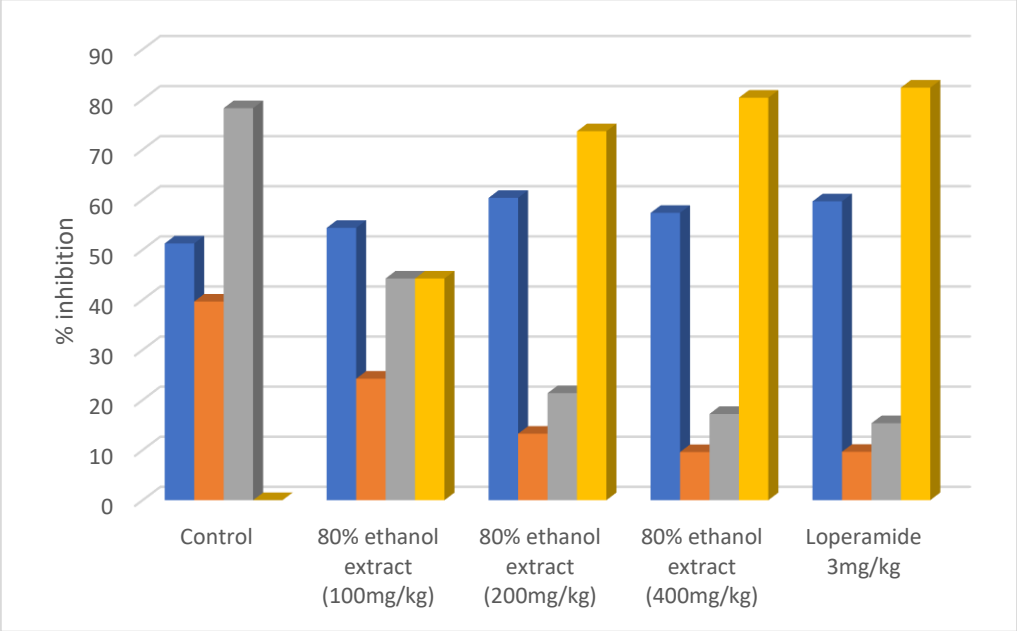

Figure 8. Gastrointestinal Transit in Mice

3.2.3 Effects of *Rubus Ellipticus* Leaf Extract on Gastrointestinal Fluid Accumulation

The findings underscore the dose-dependent efficacy of the plant extract in mitigating the castor oil-induced accumulation of gastrointestinal fluid, suggesting its potential as an anti-diarrheal agent.

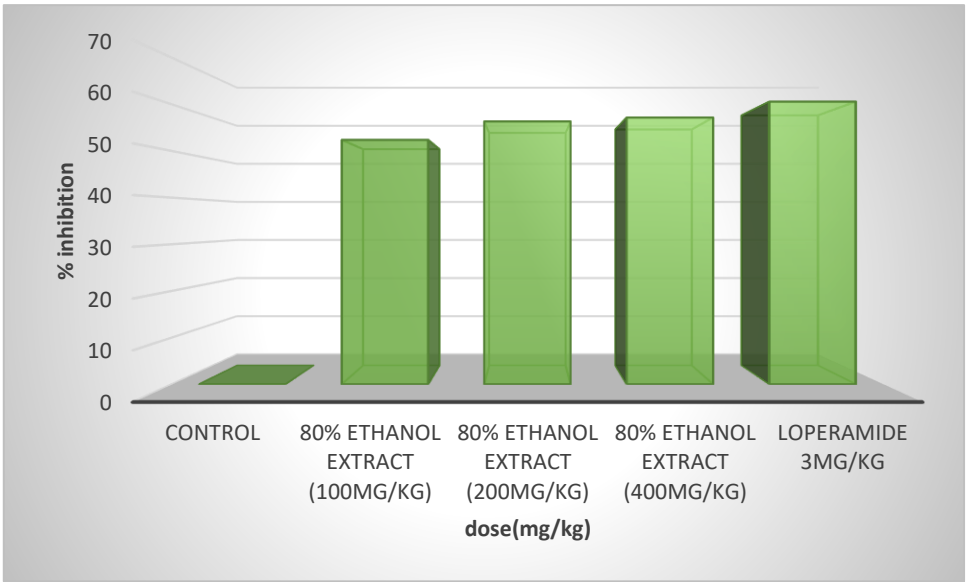

Figure 9. Gastrointestinal Fluid Accumulation in Mice

3.2.4 In Vivo Antidiarrheal Index

The plant extract had a dose-dependent antidiarrheal index, with a peak observed at 400 mg/kg, according to the results.

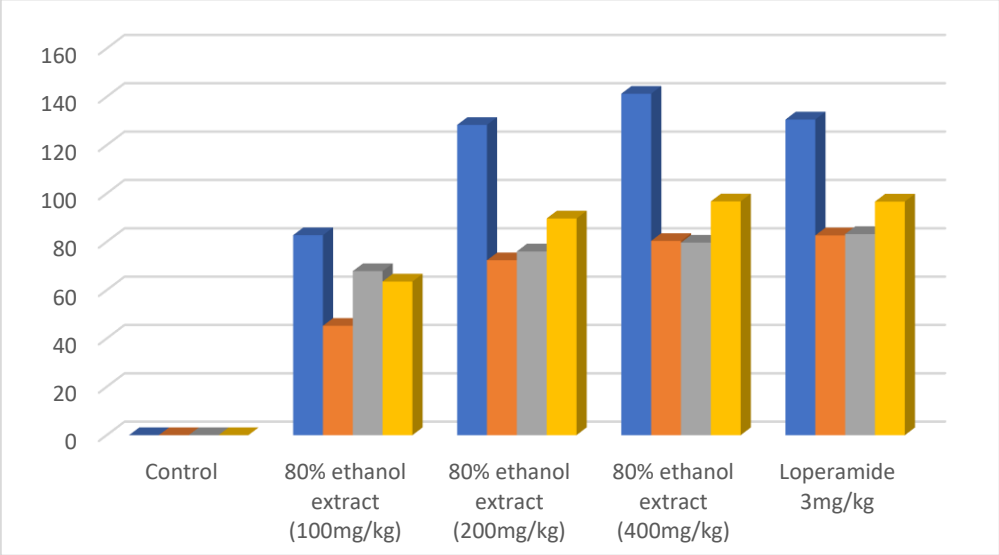

Figure 10. In Vivo Antidiarrheal Indices

4. CONCLUSION

Swiss white mice were used as test subjects to see how well the leaf extracts of *Woodfordia fruticosa and R. ellipticus* helped with diarrhoea. At all amounts tested in the experiments, the plant extract significantly delayed the start of diarrhoea, decreased the frequency of wet poop, and showed strong antisecretory effects. At higher amounts, the plant extracts also stopped the movement of animals. The study findings confirmed the conventional belief that the plant can help with diarrhoea, but more research is needed using different types of diarrhoea models and solvents.

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